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The development and assessment of novel non-invasive methods of measuring sleep in dairy cows

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ABSTRACT

Sleep is important for animal health and welfare and there are many factors, for example, the environment, illness, or stress, that are likely to have an impact on cow sleep and consequently affect their welfare. Polysomnography (PSG) is considered the gold standard for precise identification of sleep stages. It consists of electrophysiological recordings of the brain activity, eye movements and muscle activity but is costly and difficult to use with cows on farm. Accordingly, the study of sleep in cows may be limited due to the impracticability of PSG. Alternative methods of assessing sleep have been developed for humans. Some such work has been conducted for cows, but this has yet to be validated with PSG.

The main aim of this thesis was to investigate alternative methods to PSG to accurately detect sleep stages in dairy cows. Specifically, I aimed to develop a detailed 5-stage scoring system for assessing sleep stages from the cow PSG, to investigate the suitability of using lying postures and heart rate (HR) measures to assess sleep stages and to develop a model to accurately predict sleep stages using non-invasive measures in dairy cows compared with PSG.

Two studies were conducted using 6 non-lactating dairy cows in an indoor housed environment in Scotland, and outdoors at pasture in New Zealand. PSG was recorded with each cow over a period of seven days. From these data a 5-stage sleep-scoring criteria with good reliability was developed which identified two stages of light sleep, two stages of deep sleep as well as awake and rumination stages.

Video was recorded during sleep recordings and the cow's behaviour was analysed and compared with the scored sleep stages from the PSG. Some sleep stages were found to occur mainly in specific lying postures; however, overall, postures were not useful indicators of sleep stages. Heart rate (HR) and heart rate variability (HRV) were measured using a Polar HR monitor and data logging device. Differences in the HR and HRV measures were found between the sleep stages, and the patterns of these changes were similar between both Scottish and NZ cows. Finally, machine learning models were developed using supervised learning methods to predict sleep stage from the HR and HRV measures as well as the surface EMG data recorded during PSG. The models were able to learn to recognize and accurately predict sleep stages compared with the PSG scoring.

This research demonstrates that non-invasive alternatives can be used to identify sleep stages accurately in dairy cows compared with PSG. Further research is necessary with larger sample sizes and cows of various breeds and life stages; however, the success of the methods developed during this thesis demonstrates their suitability for the future measurement of sleep in cows and in the assessment of cow welfare.

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Monster


Merida


Maisel


Sassy


Dina


Paige


Mango


Millie


Ellie


Annie


Tango


Bobby Brown

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Hunter, L. B., Haskell, M.J., Langford, F.M., Connor, C.O., Webster, J.R., Stafford, K.J., 2021. Heart Rate and Heart Rate Variability Change with Sleep Stage in Dairy Cows. Animals 11. **(Chapter 4)**

Hunter, L.B., Baten, A., Haskell, M.J., Langford, F.M., O'Connor, C., Webster, J.R., Stafford, K., 2021a. Machine learning prediction of sleep stages in dairy cows from heart rate and muscle activity measures. Sci. Rep. 11. **(Chapter 5)**

Peer Reviewed Conference Abstracts

Hunter, L.B., Langford, F.M., O'Connor, C., Webster, J.R., Haskell, M.J., Stafford, K.J. (2019). Can we determine cow sleep stages from lying posture? International Society for Applied Ethology, Wellington. 21-22 November.

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INTRODUCTION

Sleep is a conserved trait among almost all animals studied to date (Ungurean et al., 2020). Birds have been found to be able to sleep mid-flight (Rattenborg et al., 2016), cetaceans sleep with half their brain while floating in the ocean (Lyamin et al., n.d.), even insects have been shown to have periods of quiet activity akin to sleep (Ho and Sehgal, 2005). There is no single definitive answer for why we humans and so many other animals sleep, however it is possible that sleep, at least in mammalian species has evolved and has continued to be expressed since it is essential for animal health and survival (Miyazaki et al., 2017). Sleep loss can have severe consequences on physical health (Faraut et al., 2012; Lasselin et al., 2015; Meerlo et al., 2008), mental health (Marques et al., 2017), relationships, and social interactions for humans (Dickinson and McElroy, 2017). As sleep architecture and patterns of sleep from brain activity in mammals seems to be highly conserved between species, there is reason to consider that sleep serves similar functions for cows as it does for humans.

There are many factors that may affect a cow's sleep, such as the lying surface, bedding type, air temperature, weather, light level, noise, and other cows. By studying sleep in cows, we could be able to determine the importance of sleep for cow welfare, and subsequently inform management practices for farmers.

Animal welfare refers to the well-being of non-human animals. This constitutes their physical health, requirements for nutrients, water, air, and space, but also their mental needs; social behaviour, sexual behaviour, play and positive affect. Animal welfare science uses various tools across multiple disciplines, such as immunology, behaviour, and physiology to try to quantify the effects of various situations and factors that might have a serious effect on an animal's welfare.

Sleep is affected by what happens to an animal and therefore could be used as a tool to assess a cow's welfare. Assuming that the functions of sleep are similar between cows and humans, experiences during the day- be they positive or negative, could influence sleep time and sleep patterns in cows. It could be possible to use changes to sleep patterns in responses to changes in the environment as a tool for understanding how these changes affect a cow's welfare (Langford and Cockram, 2010). For example, looking at the changes to sleep patterns when cows are moved to an unfamiliar environment such as a different housing or management system or when transported to an abattoir could be a way to understand how they may be impacted by the change.

The first chapter of this thesis consists of a literature review. This chapter is divided into three parts; firstly, an introduction to sleep in general, sleep physiology and sleep scoring, secondly, I reviewed other non-invasive methods used with humans and other animals for the identification of sleep and their potential for use with cows. In the final section, I present an overview of sleep research with cows and factors likely to affect cow sleep. This is where I have identified the main issue currently limiting the progression of sleep research in dairy cows and its use in welfare assessment. The gold standard method for recording sleep in humans and any animal depends on electrophysiological activity of the brain, eyes and muscles. Recording brain activity in cows is possible, but is challenging, impractical and time consuming both for data collection and analysis and is a limiting factor for future cow sleep research.

The aim of this thesis was to determine if other non-invasive methods may be used to identify the stages of sleep in cows and to develop a method that could be more easily applied on farms to facilitate sleep research in cows.

This thesis was conducted as a partnership between three organizations, AgResearch Ltd, Scotland's Rural College (SRUC) and Massey University. A year of the research program was conducted in Scotland and the rest was conducted in New Zealand. We ran two major experiments as part of this thesis, the first with indoor housed cows in Scotland, and the other with cows outdoors on pasture in New Zealand. The aim of these experiments was to record brain activity and other behavioural and physiological data simultaneously that could potentially be used to identify sleep in cows.

Chapter two describes the methods and protocols used for both trials, with a focus on the brain activity data, how it was collected, and how it was analysed and the development of a detailed sleep staging system for cow sleep.

One of the characteristics of mammalian sleep is the adoption of typical sleeping postures, and cows, unlike horses, need to lie down to sleep. Therefore, in chapter 3, we investigated the possibility of using behaviour and specific lying postures to identify stages of cow sleep. In this chapter, I have analysed the behavioural data recorded from both studies to determine if there are differences between the groups and if postures are useful for sleep stage identification in cows.

Chapter 4 examines the possibility of using heart rate (HR) and heart rate variability (HRV) as a proxy to identify sleep stages in cows. Heart rate activity changes with sleep stages in humans, we even have smart watches that claim to estimate sleep based on activity and changes in heart rate.

In chapter five, we aimed to determine if two non-invasive physiological devices could be used together to predict sleep stage accurately compared to brain activity. Using machine learning techniques, we developed and tested the accuracy of two models to predict sleep stage using heart rate and muscle activity alone.

The appendices following the main text describe supplementary data collected throughout the thesis that were not included in the chapters. Appendix A details the overall sleep results of the cows in both countries, indicating total sleep time, bout lengths etc.

Finally, I discuss the results of the thesis, the development of detailed scoring system for cow sleep, investigation of non-invasive sleep assessment measures and the implications and applications of this work for future sleep research in dairy cows.

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CHAPTER ONE

Literature Review: Measuring sleep in dairy cows and implications for cow welfare.

1 Introduction

The study of sleep in humans and in other animals is a diverse field covering a broad range of topics such as the evolutionary origins of sleep, the effects of sleep deprivation, the neurological and cellular control of sleep and the reasons and explanations for the functions of sleep. Over the past few decades, a great deal of research into sleep in a diverse range of species from tiny songbirds to walruses has been conducted, and states of sleep, or potential sleep-like states, have even been reported in reptiles (Ayala-Guerrero and Mexicano, 2008; Libourel and Barrillot, 2020), fish (Hur et al., 2012; Zhdanova et al., 2001), invertebrates (Brown et al., 2006; Medeiros et al., 2021) and insects (Hendricks et al., 2000; Ho and Sehgal, 2005)

There has been little research into sleep in cattle but interest in this field is growing. Research into the effects of lying restriction on sleep and production (Kull et al., 2019), the effects of moving into new environments (Fukasawa et al., 2018a) and diet concentrations (Fukasawa et al., 2020) on cows' sleep behaviour have been published recently. However, such research may be limited by the typical tools for studying sleep, which are impractical for use with cattle. At present the knowledge of sleep and sleep patterns of cows and the importance of sleep for dairy cow health and welfare is limited.

In this review, I will provide a brief introduction to sleep, sleep physiology and sleep recording methodology, I will review alternatives to the traditional methods for studying sleep and their potential suitability for use with dairy cows. And finally, present a general background regarding cow sleep patterns and discuss the importance of and effects of sleep loss for cows.

2 Sleep, sleep physiology and sleep scoring

2.1 What is Sleep?

Sleep is a function or a behaviour under homeostatic control, where the need for sleep builds until it has been achieved (Eban-Rothschild et al., 2017). Thus, feeling 'tired' is a signal such as hunger or thirst, indicating to an animal that it is time to sleep. Like hunger and thirst, feeling tired is then followed up with a behaviour, and instead of eating or drinking, it is characterized by a reversible state of behavioural immobility combined with reduced responsiveness (Siegel, 2005).

Animal sleep can be divided into two main stages- rapid eye movement (REM) which is a deep sleep stage characterized by a loss of muscle tone and characteristic quick bursts of eye movements, and non-REM (NREM) sleep, an amalgamation of the rest of sleep. The different states of sleep have been identified in almost all mammals studied to date (Kashiwagi and Hayashi, 2020). Interestingly, sleep timing and cycles vary remarkably between species. Some animals such as bats have been found to sleep for up to 20 hours per day and others such as elephants may sleep for only 1-2 hours per day (Siegel, 1995; Tobler, 1995). A link between body mass and sleep time has been described, with larger animals tending to sleep for less time than smaller animals and carnivores also sleeping longer than herbivores (Siegel, 2005). The question of why there is such a big difference in sleep patterns between different species and between individuals within species remains unanswered.

It is difficult to pinpoint a single reason for, or purpose of sleep and it is likely that sleep has many overlapping and interconnected functions. Many theories regarding the function of sleep have been suggested, such as the idea that sleep clears potentially harmful metabolites, the by-products of cellular function, from the brain (Xie et al., 2013). In this study, the extracellular space was found to expand during sleep and allowed these metabolites to be flushed away faster (Xie et al., 2013). Alternatively, a behavioural evolutionary theory for the function of sleep is that periods of sleep, and the associated behavioural quiescence and often protected sleeping site, would be advantageous for animals to avoid predation during a vulnerable time (Lima and Rattenborg, 2007). Regardless of the reasons for why sleep exists, it is a phenomenon that is conserved across species and shows similar or recognizable patterns, and because of this, it is likely to be extremely important for animals and their welfare.

2.2 Polysomnography

Polysomnography (PSG), uses multiple physiological signals, including brain activity, to identify sleep and sleep stages and it is considered as the ‘gold standard’ in sleep identification (Van De Water et al., 2011). PSG uses electrodes to record electrical activity of the brain (electroencephalography: EEG), the eyes (electro-oculography: EOG) and muscles (electromyography: EMG) and often also of respiratory effort (Rundo and Downey, 2019). These physiological measurements use electrodes to sense the electrical signals caused by activity of the cells in the brain, eye area and neck muscles. This information is passed to a signal amplification device. Older devices used a pen, and the signal “trace” was drawn onto a continuously moving roll of paper (Spriggs, 2015). Modern devices convert the analogue signal into a digital signal using a fixed sampling rate which can be recognized by a computer (Young, 2001).

2.2.1 Electroencephalography (EEG)

EEG is the study of the electrical activity of the brain. Depending on the data required and the area of interest, different electrode types can be used. Electrodes can be surgically implanted in the skull (Benovitski et al., 2017), on the dura (the protective lining of the brain), the surface of the brain (cortex) or within the brain (Perentos et al., 2017). These implanted electrodes and subcutaneous wire electrodes are less likely to be affected by movement artefacts on the traces (Duun-Henriksen et al., 2015; Young et al., 2006), and the signal is less attenuated or disrupted by having to pass through layers of skull, muscles, and skin to reach the electrode than surface attached electrodes. However, non-invasive cup electrodes and adhesive electrodes which are attached to the surface of the skin in the area of interest are also used in EEG but can be more easily removed or disrupted by movements, especially in non-human animal studies, and as such are more suited to shorter term recordings (Duun-Henriksen et al., 2015).

EEG in humans is often done using an electrode montage, or arrangement of electrodes. A commonly used montage is the international 10-20 method (Silverman et al., 1960), which indicates specific locations for electrodes based on defined characteristics of the skull. It is not necessary to have this many electrodes to record sleep, however a full montage allows a researcher to specify activity occurring in different regions of the brain. Recording can be conducted in what is known as a referential montage, where all electrodes are connected to a single reference electrode and the EEG activity is determined by comparing the data recorded at the electrode to the reference (Britton et al., 2016). It can also be conducted using a bipolar montage, where electrodes are referenced to other electrodes in a region. While these montage methods may have individual advantages, for example, bipolar montages are less likely to be affected by artefacts, no differences have been found between the methods when scoring sleep (Duce et al., 2014).

2.2.2 Electro-oculography (EOG)

EOG is the study of the electrical activities of the eye movements. EOG is typically conducted with non-invasive surface attached electrodes (López et al., 2016). The EOG signal is important when identifying the presence or absence of the different sleep stages and particularly for the identification of REM sleep.

2.2.3 Electromyography (EMG)

Electromyography is the study of the electrical activity of muscles (Cifrek et al., 2009). Invasive or non-invasive electrodes can be used to detect signals in the muscle of interest. There is a large body of research using skin surface EMG (sEMG), particularly in kinesiology, where muscle activity is studied during various activities and postures, in sports and movement and particularly regarding muscle fatigue (Cifrek et al., 2009). In the context of PSG, EMG helps to identify the different sleep stages as muscle activity is higher when awake or in lighter sleep, than when in REM sleep (Okura et al., 2006).

2.3 Scoring Human Sleep

Sleep is usually divided into two main stages: Rapid Eye Movement (REM) sleep and Non-Rapid Eye Movement (NREM) sleep. In 1968, Rechtshaffen and Kales published the 'manual of standardized techniques and scoring system for sleep stages of human subjects' (Rechtschaffen and Kales, 1968), which is often referred to as the 'R&K' guidelines. The R&K manual described the visual scoring of the PSG traces, and divided NREM sleep into four stages from lighter to progressively deeper sleep. The R&K manual was widely used until the American Association of Sleep Medicine (AASM) published a revised scoring system in 2007 (Iber and American Academy of Sleep Medicine, 2007), reducing NREM sleep divisions into three stages. The AASM guidelines are reviewed and updated frequently and have since become the main scoring system used around the world.

2.3.1 Rapid Eye Movement Sleep

Rapid eye movement sleep (REM) is named after the characteristic features that frequently occurs during this stage of sleep: quick, bouts of rapid eye movements, seen on the EOG trace as sharp peaks (Iber and American Academy of Sleep Medicine, 2007). Another characteristic feature of REM sleep is a very low amplitude EMG trace, or muscle atonia. In REM sleep, the brain is considered to be quite active, with low voltage mixed frequencies seen on the EEG trace that often look similar in appearance to the awake EEG. Because of this similarity, REM sleep was occasionally called 'paradoxical' sleep as the brain could appear to be awake while the patient was asleep (Peyron et al., 2020). REM sleep is particularly important for memory consolidation and learning in humans and other animals (Boyce et al., 2017).

2.3.2 Non-Rapid Eye Movement Sleep

The rest of total sleep time (TST) consists of NREM sleep. Human NREM sleep is further divided into 3 stages in the AASM guidelines, from 'lighter' stages N1 and N2 to deeper N3 sleep. In humans, N1 sleep occurs when a person is drifting off: slow rolling movements of the eyes occurs on the EOG, the brain activity begins to slow, theta waves (4-7Hz) are observed on the EEG, but the EMG remains high. The majority of TST, however, consists of N2 sleep. The EEG of N2 stage is of lower amplitude with mixed frequencies, sleep spindles- small bursts of high frequency activity often occur as do k-complexes (short high amplitude slow waves). N3 stage is often referred to as 'slow wave sleep' (SWS), as one of the defining characteristics in human sleep is the presence of low frequency delta waves (<4Hz) on the EEG. Differentiation of these stages is not typically conducted in non-human animal species although a thorough 5 stage scoring definition has been published for macaques (Goonawardena et al., 2019) and work is currently under review for mice (Lacroix et al., 2018).

2.4 Human Sleep Cycles

Typically, adult humans are mono-phasic sleepers, meaning we tend to sleep in one long bout, usually at night, but young humans and many other animals are poly-phasic sleepers, who have multiple bouts of sleep throughout the day and night (Lima et al., 2005). Generally, over the course of a night's sleep, humans cycle through the different sleep stages in a characteristic pattern, going from awake to light NREM sleep to deeper NREM sleep into REM sleep, which is usually ended by a small 'micro-arousal' and immediately back into NREM sleep (Carley and Farabi, 2016). The different sleep stages are associated with differences in awareness thresholds, which is the source of the idea of the 'depth' of sleep, as it is much easier to wake someone from REM sleep for example than a 'deeper' NREM stage (Busby et al., 1994).

2.5 Sleep restriction and deprivation

The importance of sleep is often evaluated by examining the effects of total or partial sleep deprivation on health and wellbeing. There are many studies on human sleep and sleep deprivation, or ‘sleep loss’, indicating that poor or insufficient sleep can be associated with a variety of health conditions including cardiovascular issues, obesity, diabetes, and depression to name a few (Luyster et al., 2012). Chronic sleep loss may also result in neurobiological changes that accumulate over time and may ultimately have serious health implications (Meerlo et al., 2008). In a notorious experiment, rats were placed on a small disk over a tray of water, falling asleep would result in the disk being rotated, forcing the rat to walk to avoid falling into the water (Rechtschaffen and Bergmann, 1995). While some of the effects of this study are likely due to severe stress, the rats subjected to this deprivation protocol all suffered a similar progression of negative effects on health: firstly, the development of lesions on the skin, then issues with thermoregulation and eventual death within 2-3 weeks.

2.5.1 Effects on the immune system

Sleep restriction and deprivation has effects on the immune systems in humans and animals (Luyster et al., 2012). Sleep is regulated by the hypothalamic-pituitary-axis (HPA) and parasympathetic nervous system, which play a part in the stress and immune responses of humans and other animals. Total sleep deprivation for long periods is not typically an issue facing humans and other animals, who are more likely to experience periodic sleep deprivation or restriction. Sleep deprivation has been found activate the immune system and low-level systemic inflammation, which can contribute to cardiovascular issues, and one night of recovery sleep is not sufficient for a return to baseline levels (Faraut et al., 2012). A study of sleep restriction from a typical 8 hours down to 4 hours of sleep in healthy men resulted in an increase of circulating white blood cells, an indication of immune activation, and changes in diurnal rhythm of these indicators (Lasselin et al., 2015). Immune consequences of sleep restriction and deprivation could therefore cause an increased susceptibility to infection and have many negative health consequences. Activation of the immune system also has an effect on sleep architecture, or the patterns of sleep, causing fragmented patterns and a reduction of REM sleep (Imeri and Opp, 2009).

2.5.2 Pain

A link between sleep loss and pain has been described in the literature and reviews of this topic have highlighted the bidirectional relationship of pain and sleep. Pain disturbs sleep, but equally sleep loss affects pain (Lautenbacher et al., 2006; Okifuji and Hare, 2011). Deprivation or reduction in sleep can affect the experienced intensity of pain, and many studies have found sleep deprivation to be associated with hyperalgesia (Lautenbacher et al., 2006). Much of the research in this area has focussed on the effects of REM sleep deprivation (Lautenbacher et al., 2006). A study in rats who were restricted from sleep for 6 hours per day found that chronic sleep restriction increased pain sensitivity measured by mechanical paw withdrawal thresholds, and that two days of recovery sleep was not enough to improve pain sensitivity (Sardi et al., 2018). Another study in laboratory rats suffering from sepsis due to caecal ligation and a puncture operation had significant changes to the EEG, namely an increase in NREM sleep and absence of REM sleep. They also had reduced slow wave amplitude and had highly fragmented sleep which could be an indicator of poor sleep quality (Baracchi et al., 2011).

2.5.3 Sleep Quality

Most people will have first-hand experience of the negative consequences of poor sleep, and how their sleep impacts on their quality of life. Sleep quality is a subjective experience that differs from person to person but is generally accepted to refer to the ‘restfulness’ or ‘refreshing’ qualities of the previous night’s sleep (Westerlund et al., 2014). Standardized scales such as the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) have been created to try to quantify patient reported sleep quality. In a study of Portuguese university students, subjective sleep quality measured through self-report using the PSQI was able to predict most aspects of their quality of life (Marques et al., 2017). Therefore, not only is the total amount of sleep important for humans, but the quality of the achieved sleep is also important.

The ability to measure sleep quality objectively, particularly when self-report is not possible is an issue. In humans there is a hypothesis that the spectral density of NREM sleep could be an indicator of the depth of sleep and as such a measure of sleep quality (Krystal and Edinger, 2008). Aspects of sleep architecture, such as sleep latency (SL), the time it takes someone to sleep after lying down, number of wakefulness episodes after sleep onset (WASO) and other aspects have been associated with aspects of human perceived sleep quality in some studies (Åkerstedt et al., 2016; Krystal and Edinger, 2008). While these quantitative measures are promising as a more objective way of assessing sleep quality, some researchers question the reliability of PSG derived measures for predicting the varied and subjective nature of sleep quality in humans (Kaplan et al., 2017). Most of what we know about sleep in non-humans is through studying laboratory animals as models for humans. As such, studies that assess sleep in animals to understand changes in their welfare or assess the effects of poor welfare on animal sleep are few, especially in dairy cows.

3 Alternatives to PSG for assessing sleep, and their potential for use with dairy cows

3.1 Polysomnography with cows

Due to the continuous activity of the rumen, there used to be a belief that cows and other ruminants could not achieve deep sleep (Balch, 1955). Using electrocorticography (ECoG), a method of recording the electrical activity of the brain via electrodes placed on the surface of the cortex of the brain of cows, Ruckebusch et al. (1970) identified stages of paradoxical (REM) sleep and Slow Wave Sleep (NREM). More recently, studies in calves and cows have made use of less invasive methods to record PSG using electrodes placed subcutaneously (Takeuchi et al., 1998) or on the surface of the head with non-invasive adhesive electrodes (Hänninen et al., 2008; Kull et al., 2019; Ternman et al., 2012). EEG is a less invasive method than ECoG due to the placement of the electrodes outside the skull, however the data may be more prone to artefacts, which can obscure the wave patterns from brain activity, due to muscle movement or movement of the animal. Electrodes attached to the skin of cows can be easily rubbed off and often require frequent technical attention (Ternman, 2014) and the recording equipment used for PSG is not easily applied to free-moving cows.

3.2 Miniaturization of PSG recording devices or just EEG

Recording PSG is difficult in humans and more so in animals, especially large animals such as cows being managed outdoors. Because of these difficulties, research has focussed on the development of accurate and reliable alternative mechanisms both in the engineering of logging devices and in the development of sleep-detecting computer algorithms for use in humans and other animals such as mice, rats and pigeons (Allocca et al., 2019).

A starting position to make sleep research more accessible and applicable, especially for animal research, is through the miniaturization of PSG recording devices. Small devices with electrodes implanted into the brain have been used to make long term recordings of birds in flight (Rattenborg et al., 2016). These devices have also been used with walruses (Lyamin et al., 2012), sloths (Rattenborg et al., 2008), wildebeest (Malungo et al., 2021) and many other species, however, require invasive methods for long term implantation of electrodes.

3.3 Behaviour

Sleep is driven by processes in the brain, and it has been postulated that “sleep is of the brain for the brain” (Hobson, 2005). Despite this, in some species, such as wild animals, those living in challenging environmental conditions or very large animals, it could be incredibly difficult to study sleep using these electrophysiological methods. Tobler (1995) therefore defined behavioural criteria for the identification of sleep in animals including “1) species- specific sleeping site, 2) typical body posture, 3) physical quiescence, 4) elevated arousal threshold, 5) rapid state reversibility, 6) regulatory capacity”. These behavioural criteria are useful to identify the likely presences of sleep or “sleep-like states” in animals but may not be as useful to identify specific stages of animal sleep.

Observing behaviour is the least invasive methodology for the assessment of sleep. It is also perhaps the most practical method, particularly for wild animals or where fitting animals with recording devices could be difficult. Behavioural sleep has been described for rhinoceroses (Santymire et al., 2012), elephants in zoos and in the wild (Gravett et al., 2017; Holdgate et al., 2016; Walsh, 2017), giraffes (Tobler and Schwierin, 1996) and porpoises (Wright et al., 2017), where electrophysiological methods would be difficult due to the size of the animals or their habitats. However, behaviour is an indirect measure of sleep, complicated by the possibility that animals achieve different sleep states in a variety of behavioural postures that may not be mutually exclusive.

The use of behaviour alone has been suggested as a method for identifying total sleep and sleep stages in cows. Lying and head postures of calves can be used with some reliability to predict the actual stage of sleep as analysed from their EEG (Hänninen et al., 2008). However, these same behavioural indicators of sleep could not predict sleep stages as accurately in adult cows (Ternman et al., 2014), potentially due to difficulties in differentiating between drowsing and NREM sleep, which can occur in the same behavioural postures. In calves, drowsing was not observed and therefore not scored on the EEG. This may be a reason that behaviour could be used more reliably as a proxy for sleep in calves (Hänninen et al., 2008). Behaviour alone may not be sufficient to measure and describe total sleep time and of the different stages, however, it could be combined with physiological parameters to measure the sleep of cows more accurately.

3.4 Actigraphy

Actigraphy uses accelerometers to measure movement and activity which can be used to predict sleep stages (Zinkhan et al., 2014). Actigraphy is a simple, cost effective and non-invasive method often used in the preliminary assessment of sleep disorders in humans. It is easy and low cost compared to full PSG assessments; however, it tends to overestimate sleep (Van De Water et al., 2011). The use of accelerometers to determine sleep stages has also been investigated recently in cows. Hokkanen et al. (2011) used accelerometers attached to collars to predict sleep in calves. It was validated with behavioural observations and they were able to correctly identify 82% of the occurrence of sleep, 66% of NREM sleep and 70% of REM sleep correctly. Accelerometers have also been developed to predict sleep in adult cows. The accelerometers were attached to a halter on adult cows in an attempt to use head movement and position as an indicator of sleep (Fukasawa et al., 2018b; Klefot et al., 2016). These methods have shown good accuracy in predicting sleep; however, they have only been validated using lying behaviour, head positions and eye closure, which may not be as true a representation of the actual sleep experienced that could only be reliably quantified with PSG.

Behavioural estimates of sleep tend to overestimate total sleep and sleep stages in cows (Ternman, 2014) and as such may not yield the most accurate results and cannot be used as a gold standard. Actigraphy has the potential to be used as a measurement tool for calves and cows, however more research, and importantly, validation with PSG is required.

3.5 Physiological Covariables

As mentioned earlier, human PSG can often contain several physiological measures including ECG and respiratory effort (McCarley, 2007). Sleep is intertwined with the autonomic nervous system (ANS) and as such, transitions to sleep stages also influence the interrelated systems such as the cardiovascular, respiratory, and metabolic systems, impacting the heart rate (HR), respiration rate and body temperature.

HR and heart rate variability (HRV), the time between successive heart beats, can be used to identify sleep in humans (Chouchou and Desseilles, 2014). Methods using machine learning, the development of computer programs to learn from data, have been developed to predict sleep stages accurately from HR and HRV metrics alone (Mitsukura et al., 2020; Xiao et al., 2013). Changes to the HR and HRV have also been found to occur during sleep in dogs (Varga et al., 2018).

HR and HRV has been recorded in cows, however these were shorter term recordings used to evaluate the effects of stress (Kovács et al., 2016; Mohr et al., 2002; Stewart et al., 2016) or pain (Byrd et al., 2019; Stewart et al., 2008). HR measurement can be conducted with dairy cows using non-invasive surface electrodes and so offers a viable opportunity for this methodology to be applied to sleep assessment in cows.

Regulation of core body temperature occurs during NREM sleep; however, it is reduced during REM sleep (Troynikov et al., 2018). Core body temperature fluctuations are more challenging and more invasive to record in dairy cows and require invasive methods such as internal vaginal (Vickers et al., 2010) or rectal sensors, subcutaneous implantation of sensors (Lea et al., 2008), rumen boluses, or ear temperature sensors (Rutten et al., 2017). Non-invasive temperature monitoring using thermal imaging has also been developed with cows to assess surface body temperature (Salles et al., 2016). Eye temperatures may be a better indicator of core body temperature (Church et al., 2014). However, these can be influenced by the environmental conditions such as air temperature and wind speed (Bell et al., 2020) and could be impractical to use for long term recording. Body temperature could be an interesting factor to investigate during sleep in dairy cows, however, is not likely to be useful as an alternative to PSG.

Respiratory effort, including parameters such as respiration frequency, respiration regularity as well as respiratory depth can be used to identify sleep in human subjects (Long et al., 2014), particularly when combined with HR and HRV signals (Ebrahimi et al., 2015). Measuring respiration rate in cattle is often used in assessment of heat stress and can be calculated by timing breaths manually using a stopwatch (Schütz et al., 2014) or from infrared thermography (Stewart et al., 2017). More recently, an algorithm has been developed to identify respiration rate in dairy cows using deep learning and video analysis with a mean accuracy of 93% (Wu et al., 2020). Respiration rate could be a potential non-invasive method to assess sleep in dairy cows, however, as with human sleep, it may be more effective in combination with other non-invasive measures.

There are a few possibilities for alternative technologies to use as a proxy compared to PSG for assessing sleep and sleep stages in cows. Behavioural based methods, including the observation of lying postures and actigraphy have been previously used with cows, however, need further validation with PSG. Alternative physiological methods, particularly HR may potentially be a useful proxy, however further development and validation is needed.

4 Sleep research in cattle and the importance of sleep for cow welfare

4.1 Scoring sleep in cows

Ruckebusch et al. (1970) observed and defined the two main stages of sleep in cows; slow wave sleep (SWS) aka NREM sleep characterized by high amplitude low frequency wave patterns, and paradoxical sleep (PS) aka REM sleep characterized by high frequency low amplitude wave patterns similar to the awake pattern but accompanied by a lack of muscle tone and rapid eye movements. In addition, Ruckebush et al. (1970) also defined two states of wakefulness in the cow: Active Wakefulness (AW) and 'Drowsiness' (DR) an intermediate stage between active wakefulness and slow wave sleep exhibiting a mix of fast and slow wave patterns. Although defined as a state of wakefulness in cows, this definition is very similar to human stage N1 or N2 sleep, which is often classified as light sleep. Indeed, more recent studies of sleep in calves and cows which have scored sleep using the Ruckebush and R&K guidelines have also scored ‘drowsing’ but indicate it to be an ‘intermediate’ stage (Ternman et al., 2018).

4.2 The cow sleep cycle

Cows housed in a tie stall barn were found to sleep for approximately 4 hours per day (Ruckebusch et al. 1970), with approximately 3.5 hours in NREM sleep and only about 30-45 minutes in REM sleep. Humans typically sleep in a single long bout at night and are considered to be monophasic, cows however, sleep in several shorter bouts about 8-9 times per day, with the majority of these bouts occurring during the night. Cattle are considered to have a polyphasic sleep cycle (Ruckebusch et al., 1970; Ruckebusch, 1975a).

The majority of cow sleep consists of the NREM stage or ‘deep sleep’ which, according to Ruckebusch et al. (1970) occurs in bouts of approximately 15-20 minutes in length followed by bouts of REM sleep around 5 minutes in length. These are usually followed by either awakening to AW or DR or another bout of NREM sleep. In a study conducted using non-invasive PSG methods, Ternman et al. (2012) also reported REM sleep bout lengths of 3 ± 1 minutes. In contrast, Ternman et al. (2012) reported average NREM sleep bouts of only 5±3 minutes from the 8 cows included in their study which was markedly shorter than the bout lengths reported by Ruckebusch et al. (1970). The observed differences in bout length could be due to the differences in analysing the wave patterns between the two studies, the experience of the EEG scorer or differences in the technological methods used for recording. Ternman et al. (2014) noted that the electrodes and equipment on the cows needed to be adjusted frequently in their studies as the cow’s movements caused them to be displaced. This frequent disruption to the cows may have affected their sleep bout lengths. Additionally, the outfitting of cows with these devices may have also shortened their sleep bouts due to discomfort from the wires and other attachments, however Ternman et al. (2018) found no significant effect of the sleep recording devices on the first night compared to subsequent nights in dairy cows.

4.3 Factors that may affect cow sleep

4.3.1 Environment level factors

The sleeping and resting environment is likely to have a pronounced impact on the sleep of cows. Human sleep can be affected by comfort, temperature, noise, light and novelty. Cow sleep can probably be affected by these factors as well.

4.3.1.1 Housing type

Toutain and Ruckebusch (1973) found that cows outdoors rested their heads for about half the amount of time (22.5±10.7 minutes) compared to cows housed indoors (42.9 ± 10.8 minutes). They had previously associated this behaviour with REM sleep (Ruckebusch et al., 1970). When the cows returned indoors after a period of time on pasture, they showed a significant rebound in the time spent with head resting (107 ±13 minutes) which took 4 weeks to return to the baseline level for indoor housing (43± 7 minutes). The data from the ECoG of a cow on pasture and in a stalled environment showed a similar effect and the total sleep time (NREM & REM sleep) of the cow on pasture was only 2.5 hours which was significantly less than when stalled (Ruckebusch et al., 1974). The cow on pasture also tended to have a more polyphasic pattern of sleep with short periods of sleep in the morning and afternoon following grazing periods. Housed cows had more of a monophasic sleep pattern with almost all sleep occurring during the night (Ruckebusch et al., 1974; Toutain and Ruckebusch, 1973).

Tucker et al. (2007) found that housed cows lie down with their head resting on the ground or tucked on the flank twice as much as cows maintained outside. They also found that cows outside during the winter lay down significantly less than those housed indoors, and that cows outdoors lay down more often in a tucked posture, possibly to preserve body heat. This could suggest that temperature and weather are likely to influence the postures adopted during sleep and potentially the pattern of sleep stages of cows.

The differences in the amount of sleep shown between cows housed or held outdoors begs the question regarding the minimum sleep requirements and the impacts on the welfare of cows on pastures if they are exhibiting such a comparatively low amount of sleep. It could be possible that cows on pasture are able to achieve a better quality of sleep and as such, need less of it to feel refreshed or that they can become more efficient sleepers than those indoors, and they may meet their sleep requirements in a shorter period of time. It is also possible that such a short period of sleep such as the 2.5 hours exhibited by cows on pasture (Ruckebusch et al., 1974) is all that is required, and the higher amounts achieved by indoor-housed cows is a form of 'luxury' allowed by the security and food availability of a housed environment.

As discussed above, cows on pasture have been found to exhibit shorter lying times than cows indoors, however Krohn and Munksgaard (1993) found that cows on pasture exhibited significantly more lying with the head resting than cows in indoor tie-stall housing. Interestingly, Krohn and Munksgaard (1993) also found that cows on pasture exhibited significantly more resting bouts where lying with the head resting on the flank occurred, and the duration of these bouts was significantly longer. This could suggest that despite shorter total lying times, cows may be able to condense their sleep bouts to achieve sufficient sleep.

Charlton et al. (2011) found that cows with access to pasture as well as an indoor free-stall area, chose to lie down for 90% of their lying time indoors. However, when they investigated the motivation for pasture access in dairy cows, they found that the cows in their study would spend approximately 80% of the night outdoors and were highly motivated and would walk longer distances (260m) to access a pasture at night (Charlton et al., 2013). As sleep was found to occur mainly at night (Ruckebusch, 1972) these findings could also indicate a preference for cows to sleep at pasture. It is unclear though, if this preference for pasture is due to intrinsic features of the outdoor environment, or difference in space and therefore ‘comfort’ in being able to stretch or engage in more expansive lying postures. Shewbridge-Carter et al. (2020) found that cows chose to lie down on a larger surface area of their less preferred bedding surface type when the space of their preferred surface type was reduced by the addition of a metal stall. Therefore, sleep in cows could be affected by the amount of space available for them to lie down in.

4.3.1.2 Lying Surface and Quality

Dairy cows sleep lying down (Ruckebusch, 1975a) unless forced to stand, or when not properly adapted to the environment. Because of this, factors that affect cow lying behaviour are also likely to influence cow sleep as well. The lying surface is important for cows. Cows have been found to spend longer lying in stalls with more bedding than in sparsely bedded stalls (Tucker et al., 2009), and the quality of the bedding affects lying time as well. Cows will spend less time lying on wet bedding than on dry bedding (Fregonesi et al., 2007b) but also find dirty bedding to be more aversive than wet bedding alone (Schütz et al., 2019). Unsurprisingly, lying surfaces are also important for cows managed outdoors. A study of cows and heifers managed in outdoor enclosures found that in muddy and wet enclosures, they would lie down for as little as 3.2 hours per day and would prefer to lie on a concrete apron rather that to lie in the mud (Chen et al., 2017). Such a short lying time would severely limit the amount of sleep that could be achieved significantly affecting the welfare of the cows.

4.3.1.3 Familiarity of the environment

A study investigating collapsing behaviour, which can be dangerous and cause severe injuries, during sleep in horses, found that 50% of the horses with a history of collapses had experienced a stressful situation such as a barn relocation or were housed improperly (Fuchs et al., 2017). In several of the cases the collapsing issues improved when the issues of housing were addressed, and it is hypothesized that some horses were uncomfortable in a new, unfamiliar, stressful, or inadequate environment and as such were reluctant to lie down and sleep. Consequently, when they transitioned into REM sleep, they collapsed, and showed the onset of muscle atonia characteristically associated with REM stage sleep (Fuchs et al., 2017). Similarly, in a study of behavioural REM sleep in zoo giraffes, relocation significantly affected the pattern and amount of REM sleep (Sicks, 2016). As this change to the REM sleep cycle was likely due to the stress of transport and of unfamiliarity with the new environment, the authors concluded that changes in sleep patterns could be a useful indicator for mental state and overall welfare.

In Japanese cows, sleep estimated from lying posture was lower on the first day after moving into a new environment and took nine days to stabilize (Fukasawa et al., 2018a). Dairy cows are not transported on shorter trips as frequently as horses. In some systems, however, such as with sharemilking in New Zealand, where dairy farmers may own the cows but not the land or facilities used to graze and milk their cows (Stafford, 2017), whole herds of cows are moved to new properties more frequently. The change of environment and experience during transport could therefore have an effect on their ability to sleep. The most common reason for transporting cattle, however, is transport to the abattoir. A study of cull dairy cows in Canada found that following transport, cows spent an average of 82±46 hours in the market system before being slaughtered (Stojkov et al., 2020). Stress from transport as well as unfamiliar environments among other factors are likely to affect a cows’ sleep during this time.

4.3.2 Cow level factors

4.3.2.1 Pregnancy, Parturition and Parity

Pregnancy and parturition are physically demanding processes for cows and are likely to affect their sleep. Cows showed a significant decrease in the total amount of sleep in the 24hrs prior to calving, and there is some evidence indicating a significant decrease in the amount of NREM sleep in the week after calving. However, this is based on data reported from a single cow (Ruckebusch, 1975b).

The stage of lactation has also been found to have an effect on sleep in cows. Cows in the late dry period and also late pregnancy were found to exhibit more REM sleep, and cows in early lactation exhibited less REM sleep than during other stages of the lactation cycle (Ternman et al., 2019). Lying time has been found to increase with days in milk but is shorter in cows with higher milk yields, presumably due to the increased energy demand and need to eat (Norring et al., 2012; Stone et al., 2017). High-yielding cows had a shorter latency to become inactive without ruminating and with the neck relaxed after lying down, which may demonstrate an ability to get to sleep faster in order to achieve sufficient sleep even though total lying time is shorter (Norring et al., 2012).

Stone et al (2017) found that primiparous cows were faster to lie down without ruminating and the neck relaxed compared to multiparous cows. There is some evidence that a longer latency to sleep onset is associated with a subjective experience of poor sleep in humans (Åkerstedt et al., 2016; Westerlund et al., 2014). Although the use of neck activity as an indicator of sleep may not be a particularly accurate method to quantify the actual awareness state of cows, these results may indicate that age is an important factor that may affect the normal sleep cycles or sleep quality. Age is known to affect total sleep time and patterns in humans and other animals. Sleep patterns change with increasing age in rats (Kirov and Moyanova, 2002) and young mammals often exhibit more total REM sleep which slowly decreases to an adult level with development. This is particularly apparent in altricial animals (Siegel, 2005). The EEG wave patterns, and frequency spectrum of calves change from birth to ten weeks of age (Takeuchi et al., 1998) and the stage of drowsing was not observed in dairy calves in the study by Hänninen et al (2008). It is possible that calves do not drowse as older cows do and age is therefore an important consideration for the study of sleep in cows.

4.3.2.2 Social stress

Mice subjected to chronic social stress after being placed in a cage with an aggressor, showed a decrease in the power spectrum of the EEG during Slow Wave Activity (SWA: deep NREM sleep) during recovery sleep after a short sleep deprivation period of four hours (Olini et al., 2017). Decreased power of SWA is thought to be a potential measure for poor sleep quality and the EEG power could be indicative of the depth of sleep achieved (Krystal and Edinger, 2008). Cows can experience social stress from several sources including overstocking and social group rearrangement. After regrouping, dairy cows had a tendency to show a reduction in lying time and had fewer lying bouts than before regrouping (von Keyserlingk et al., 2008). There is evidence which shows that overstocking reduces lying times in dairy cows, but it is difficult however, to tease out how much of this is caused by the lack of suitable lying places or social competition alone (Fregonesi et al., 2007a). As social stress affects lying time, it also is likely to have effects on the sleep of cows.

4.4 Implications for sleep and cow welfare

4.4.1 Sleeplessness/Sleepiness

Sleeplessness is an unpleasant subjective experience for humans. It is possible that other animals may experience a similar sensation when they do not achieve the required amount of sleep. Humans subjected to periods of repeated short sleep restriction reported that sleep restriction was not overly stressful per se but rather ‘burdensome’ (Simpson et al., 2016). Ruckebusch et al. (1975b) commented that the sleep deprived cows seemed to be ‘irritable’ towards the farm staff and experimenters. While this observation may be based on subjective observations and could be an anthropomorphism, it is possible that these observations are a valid description of the experiences of the cows at that time. Occasional experiences of sleep restriction and deprivation may be manageable over time, however chronic or repeated sleep deprivation could lead to the very negative feeling of exhaustion. The strong motivation for cows to lie down (Metz, 1985) and the significant rebound and almost doubling of sleep time after deprivation in cows as well as preference to sleep when time available to eat was also restricted (Ruckebusch, 1974), indicates that sleep is extremely important to cows.

Ruckebusch (1974) found that REM sleep deprivation resulted in a slight increase in the amount of NREM sleep but also a severe fractionation of NREM sleep bouts into small ‘micro-cycles’ of 2 minutes in length compared to a typical duration of 18.9 minutes when not restricted. Fractionation of sleep can be associated with poor sleep quality in humans (Krystal and Edinger, 2008). As sleep typically occurs during the night (Ruckebusch, 1975a), lying restriction over-night would likely affect the sleep patterns of cows. Fisher et al. (2002) found that periodically depriving cows of lying from 15:00h to 6:45h over a period of 7 days was associated with increased plasma cortisol and reduced responsiveness to corticotropin releasing hormone (CRH).

The experience of insufficient or a total lack of sleep could be a welfare concern for dairy cows. Sleep is included under the behaviour section of the ‘five domains’ model for animal welfare (Mellor and Beausoleil, 2015) with exhaustion as an associated negative mental state and feeling refreshed or energized associated with a positive state. More research on sleep patterns and particularly on minimum sleep requirements for cows is needed to better inform management decisions regarding their resting behaviour and sleep on farms.

4.4.2 Sleep and health

As discussed previously, sleep has a significant relationship with health, the immune system and pain. It is therefore important to understand the sleep requirements of cows and the impacts that the environment can have on sleep for their physical health and resilience to potential illness and disease.

Lameness is a highly prevalent (Rutherford et al., 2009) and significant cause of pain experienced by dairy cows, particularly those in indoor housing systems (Haskell et al., 2006). During a long-term study of cow sleep in 1975, a cow developed an interdigital abscess, and was treated during the study. This unexpectedly allowed for the opportunity to investigate the effects of pain/illness on the sleep of cows. Ruckebusch (1975b) found that this cow showed increased fractionation of the periods of slow wave (NREM) sleep and fewer periods of paradoxical (REM) sleep during inflammation. After treatment he observed increased slow wave sleep bouts and more episodes of paradoxical sleep in the cow which he associated with relief from pain.

Another common source of pain in dairy cows is mastitis, an infection and inflammation of one or several quarters of the udder. Mastitis has also been found to reduce the lying time of dairy cows (Fogsgaard et al., 2012; Medrano-Galarza et al., 2012; Siivonen et al., 2011) and there is some evidence to suggest they prefer to lie on the side with the unaffected quarter, probably due to the pain associated with lying on the inflamed side (Siivonen et al., 2011). Pain from mastitis and reduced lying time might also affect the amount of sleep and sleep quality of cows. The immune consequences of sleep loss may also aggravate the issue.

4.4.3 Sleep in welfare assessment

Changes to the sleep patterns of animals in response to changes in their environment can be potentially used as a tool for the assessment of animal welfare. Disruptions to sleep can be caused by emotional experience in humans and it follows that sleep disturbances in animals could perhaps also be due to their emotional or mental state in response to their experiences (Langford and Cockram, 2010). Sleep patterns were affected by transport and change of environment in horses and giraffes (Fuchs et al., 2017; Sicks, 2016) and there are many environmental and individual factors that have the potential to affect the experience of sleep in cows. More research is required to update our knowledge of the typical sleep patterns of cows before sleep could be used as an indicator of mental state in cows. However, measuring changes to sleep could be a useful tool to assess the mental state of cows in many situations and could be beneficial for future welfare assessment.

5 Conclusion

Sleep is an essential function for the health and welfare of humans and animals. It plays an integral role in body functions such as metabolism and the immune system, and the loss or restriction of sleep can have significant consequences on health and well-being. There are many external and individual factors that have the potential to affect a dairy cow's sleep, including the housing environment, temperature and weather conditions, pain, illness, and stress, and thus have an effect on her health and overall welfare. Therefore, I believe sleep is an essential function that should be considered in all management decisions for cows on farms. Further research is needed to investigate cow sleep requirements and to understand more regarding its impacts on and importance for cow health and welfare. Development of capability in sleep measurement could be used to assess the effects of changes in the environment by differences in sleep duration and patterns. PSG is the gold standard method to identify sleep and the sleep stages from light to deep sleep in all animals as it is a direct reflection of the brain and physiological functions involved in the regulation of sleep. However, PSG, is expensive and difficult to use, and is therefore a limiting factor for future studies of the effects of sleep for cow welfare. There are several possible proxy methods that have been developed to study sleep in humans and dairy cows based on sleep behaviour or physiology, but that may also not be feasible for used with cows or require further validation. Accordingly, more research is required to develop more reliable and practical methods to assess sleep stages in cows validated with PSG to facilitate future research on sleep in cows.

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CHAPTER TWO

Cow Polysomnography and Sleep Scoring Methods

The aim of this chapter is firstly to describe the PSG recording methodology used to record cow sleep in the two main experiments that are presented in this thesis from cows housed indoors in Scotland, and cows managed outdoors on pasture in New Zealand. The second aim is to present an updated and detailed visual scoring criteria for five stages of vigilance (Awake, N1, N2, N3, REM) for dairy cows developed using a combination of human scoring, previously published guidelines and observations from the collected data set.

Methods

Animal Ethics

Approval was obtained for the methods and manipulations used in the research conducted in Scotland from the UK Home Office (Project Licence P204B097E) and SRUC Animal Ethics Committee (Ref. ED AE 03-2018). Approval for the methods and manipulations of the trial work conducted in New Zealand was obtained from the Ruakura Animal Ethics Committee (AE 14708).

Scottish Cows

Six healthy non-pregnant, non-lactating cows, known by farm staff to be friendly and that were due to be retired for the herd due to issues with fertility were selected from the Acre Head Research Farm, Dumfries, Scotland. The cows were housed indoors in a large deep-bedded straw pen within the main barn and were managed as per typical farm practice. During recording the cow was penned individually in a 4x4m pen separated from the other cows by a 2m buffer zone which allowed visual and auditory contact with them to minimize potential disturbances to recording equipment (Figure 1).

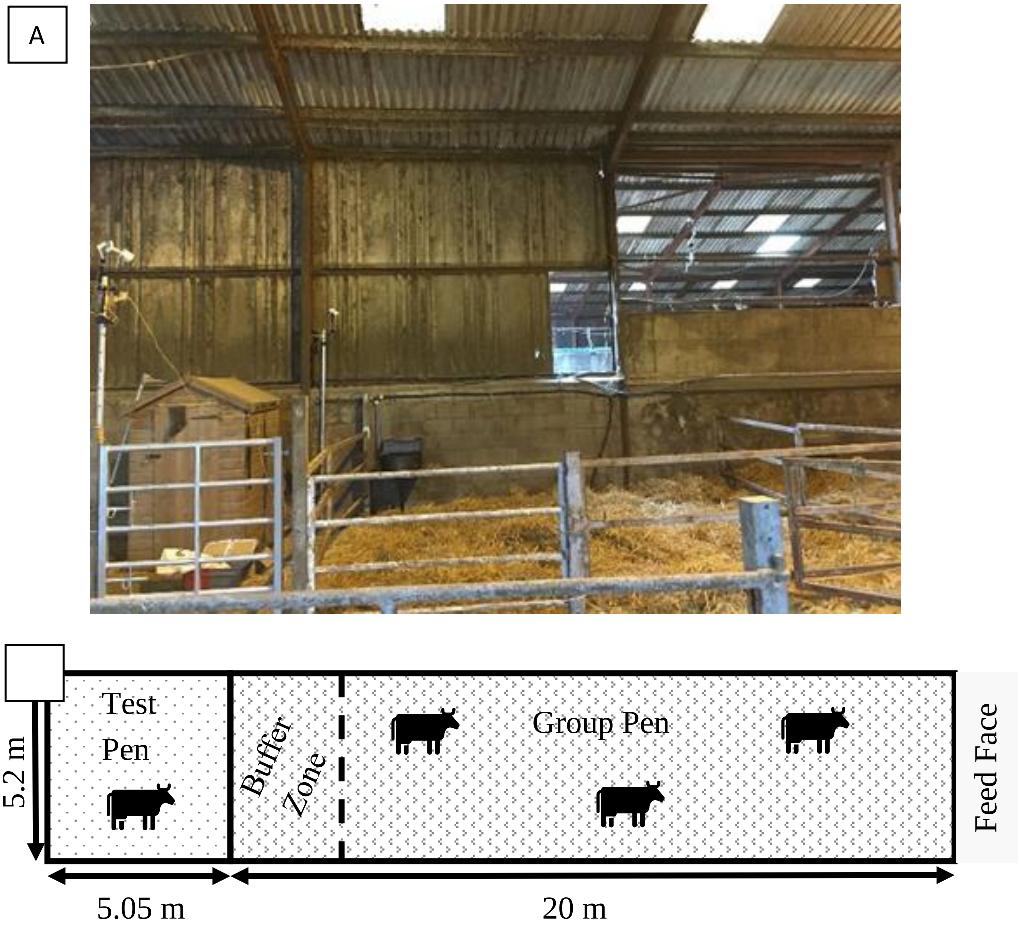


Figure 1. The Scottish housing design (A) and (B) a diagram of the test pen, buffer zone and group pen configuration.

New Zealand Cows

Six healthy pregnant, non-lactating, cows from the herd at Dairy NZ Lye Farm, Hamilton, New Zealand were selected based on their known friendly and calm behaviour towards humans. The cows were managed in a large (44m x 21m) outdoor area created with electric fencing within a large paddock. During recording, the target cow was moved into a 10x10m pen created with non-live electric fencing tape and surrounded by a 2m buffer zone fenced with live tape separated from the group area (Figure 2). The buffer zone between non-live and live wires was created to reduce potential noise on the electrophysiological traces while also minimizing possible disturbances to recording equipment from groupmates. The fencing configuration was moved around within the paddock as the ground became too wet or muddy. All cows were allowed to graze and supplemented with silage regularly.

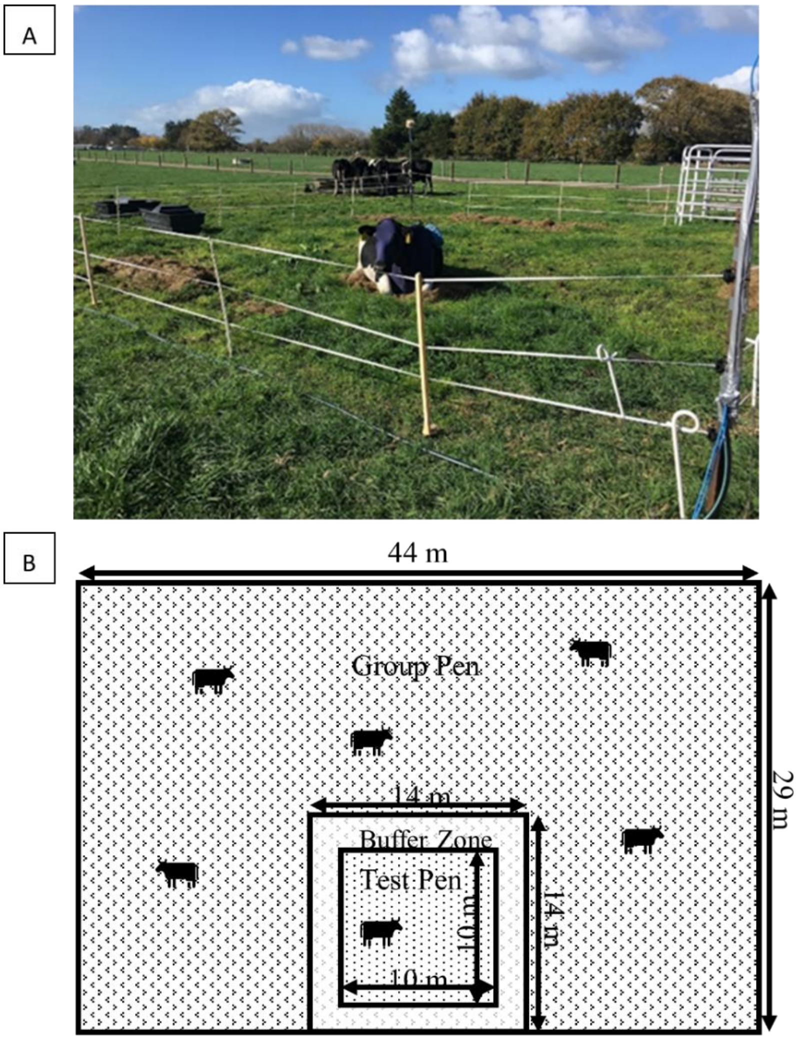


Figure 2. The New Zealand test pen created with non-live electric fencing tapes with a cow being recorded and groupmates eating silage in the background (A) and diagram of the New Zealand test and group pen configuration (B)

Training Procedure

Considerable training and habituation were conducted with the cows in both Scottish and New Zealand studies prior to the start of data recording (described below in Table 1). This habituation was done to ensure the cow was not fearful of the researchers or materials and to minimize stress to the cows during device attachment and recording. The training and habituation were also conducted so maintenance of the equipment and devices could be done with the cow in the test pen, avoiding the need to put the cow through a race or crush to fix or adjust the device attachments which could be more stressful to her.

The Scottish cows were initially moderately avoidant of human contact, and several weeks training was required to build positive relationships with each animal so that they allowed people to approach them, being touched, and handled particularly on the head and neck. The New Zealand cows were selected from a group of cows who had previously been involved in research trials and had experience from a very young age of being touched and handled by people. We selected cows for the New Zealand trial that could be approached and touched in the pasture, thereby cutting down on the amount of habituation time required.

A checklist was created to monitor each cow's progress until they were fully prepared for the recording (Table 1). Positive reinforcement with food rewards (concentrate/pellets) were given to reinforce desired/good behaviour.

Halter training was conducted with each cow, firstly to habituate her to being touched around the face, but also to stay still during device attachment. The New Zealand cows were halter trained with rope halters over the span of 2-3 weeks according to Dairy NZ's research technician SOP for halter training. The cows were then habituated to the sounds and smells of the materials and devices and to wearing the protective gear. This gear included an elastic surcingle girth, and a Lycra horse hood, modified to fit the position of the cow's ears. The cows were habituated to the sound and feel of battery-operated hair clippers. The Scottish cows were also habituated to the smell of hair removal cream (Veet extra sensitive, UK). Training depended on the individual cow's personality and took varying amounts of time depending on the individual cow's behaviour

Once the cows had successfully passed the habituation criteria and were ready for recording, they were moved into the test pen. They were held there for 24 hours to become familiar with the new environment prior to device attachment or recording as well as providing a baseline for the cow's normal lying behaviour prior to being outfitted with the recording devices. The ethical guidelines (ED AE 03-2018) restricted housing individually for a maximum of one week.

Table 1. Steps for training and habituation of dairy cows for attachment of recording devices and protective gear

Behaviour	Description
Approach cow	Approach the cow by slowly moving closer to the cow and the edge of her flight zone over several days until she was accustomed to the presence of people and would allow a person to be next to her.
Touch face	Touch all over her face head and ears, initially with one hand, then both.
Touch Body	Pat and stroke the neck, back, rear and around the girth of the cow.
Halter on (Scottish cows only)	Introduce the cow to the smell, sight and sound of the halter and have her allow it to be put on. See the halter hanging on the fence, touch the halter with the nose, put nose through nose band, wear unlatched halter for a short period of time, wear latched halter for a short period of time, wear halter for a long period of time.
Tying to the gate/lead training	Introduce the cow to the lead rope, train the cow to stand when tied to the side of the pen or to walk beside the researcher when being led.
Rope halter training (New Zealand cows only)	Introduce cows to rope halter, halter and loosely wrap the rope around a fence to provide some resistance, eventually tie the rope to the fence for a short period of time.
Clippers	Sniff clippers (when off), rub on face and neck. Turn on clippers and rub on face and neck (without clipping hair)
Hood	Introduce the cow to the sight, smell, sounds and sensation of the horse hood. Teach the cow to allow it to be placed over her head and secured, see the hood and movement of the fabric, smell the hood, rub the hood on the neck, body and face and over the eyes, put nose into nose hole, wear non-zipped hood around the neck, attach hood over ears and eyes and zip up, wear hood for long periods.
Hair Removal Cream (Scottish cows only)	Introduce the cow to the smell of the hair removal cream. Test a patch on the neck prior to removing hair on the forehead, above the eyes and the neck.

Attachment of devices

Hair removal

The boundaries of the electrode attachment zone were two lines vertically from the lateral canthus of the eyes, a horizontal line just above the orbital bone above the eyes and a horizontal line just below the poll at the top of the forehead (Figure 3a). The hair in this main area and at the at the other electrode attachment sites, the lateral side of the eye and the trapezius muscle of the neck was removed immediately prior to electrode attachment using clippers and/or hair removal cream (Veet extra sensitive, Reckitt Benckiser UK, for the Scottish cows only). The clipped skin was then wiped clean and sanitized using alcohol wipes. Hair removal cream was used initially as it is dissolves hair down to the skin. Clippers leave an amount of stubble which could cause issues for electrode impedance (skin-electrode contact). Most of the Scottish cows were accepting of this cream, however some found it particularly aversive, probably due to its strong smell, and had a strong negative behavioural reaction to it. Hair clippers were then used on these cows, and while not removing hair as closely as the cream, good impedance values were still obtained. Hair removal for the New Zealand trial (conducted after the Scottish trial) was done using clippers only.

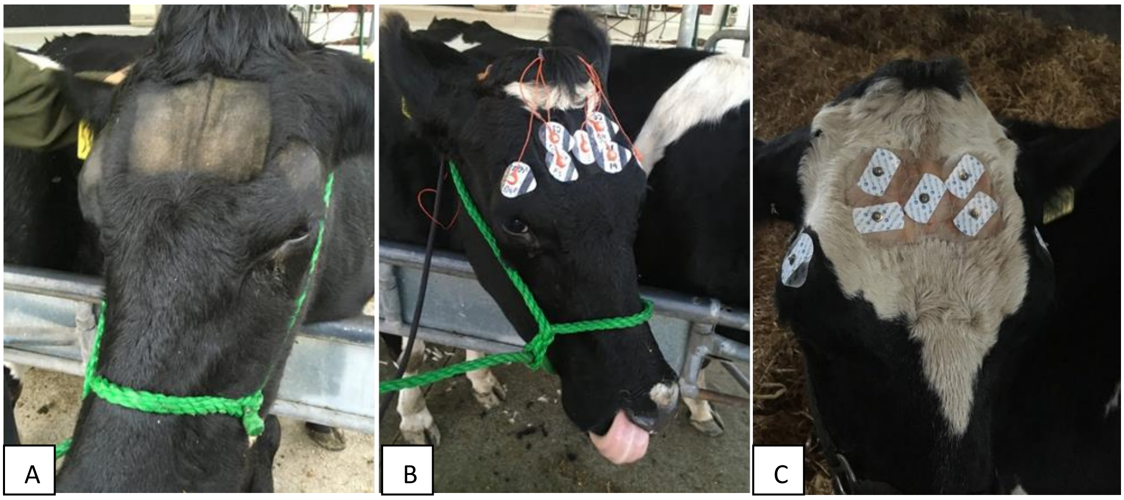


Figure 3. [A] Hair clipped at electrode location, [B] Electrodes attached, and snap leads connected [C] Adhesive electrode placement for EEG and EOG.

Electrode attachment

Electrodes were placed as previously described in studies of sleep in dairy cattle (Hänninen et al., 2008; Ternman et al., 2012). Due to the cranial anatomy of dairy cows and the presence of many sinus cavities, a typical electrode montage as is used in human studies that records all regions of the brain is impossible. The electrode placement described is the most suitable option to reduce brain signal attenuation resulting in better quality data. This placement involves a reference electrode places in the centre of the attachment zone, surrounded by four EEG electrodes in a box formation around the four corners (Figure 4). An EOG electrode was placed on the lateral side of each eye, and two EMG electrodes were placed approximately mid-way along the trapezius muscle in the cow’s neck. Finally, a patient ground (PGND) electrode was attached behind the poll at the top of the cow’s head.

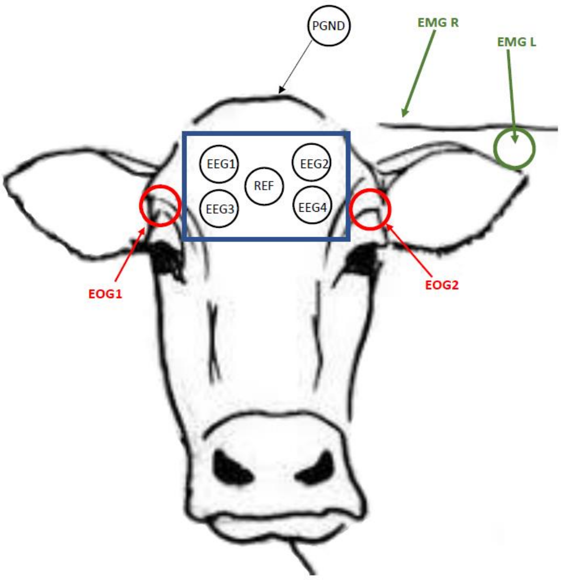


Figure 4. Electrode placement diagram

Ag/AgCl cup electrodes:

The Initial recordings in the Scottish cows were made with Ag/AgCl cup electrodes (Embla Systems, Kanata, Canada) commonly used for recording the EEG of humans (Figure 5a). The cups of the electrodes were filled with ultrasound gel or Ten20 conductive neurodiagnostic electrode paste (Weaver and Company, Aurora, USA) to improve conductivity of the signal and attached to the cow using cyano-acrylate (Loctite 454 or Loctite gel control, Henkel Corp., Dublin, Ireland or Gorilla Glue gel, Gorilla Glue Inc., Cincinnati, Ohio, USA) placed in small dots around the outside edge of the electrode and held in place on the cow's head for 10 seconds and secured with a piece of adhesive tape. Recordings from this type of electrode were of good quality however, due to the small surface area for attachment with glue, could be easily scratched off or pulled off with the cow's normal behaviour or from rubbing on features in the pen.

Single-use pre-gelled adhesive ECG electrodes with clip on leads

After encountering issues with the Ag/Cl cup electrodes, single use adhesive snap electrodes (Natus disposable LM solid gel electrodes, Embla Systems, Kanata, Canada) were trialled as an alternative and were preferred due to the larger adhesive surface area. Several dots of glue could be spread over the entire adhesive patch allowing for a more secure hold. These electrodes could be used for several sequential recording sessions and wire leads could be snapped on and off easily and required less maintenance than the cup electrodes. (Figure 5b).

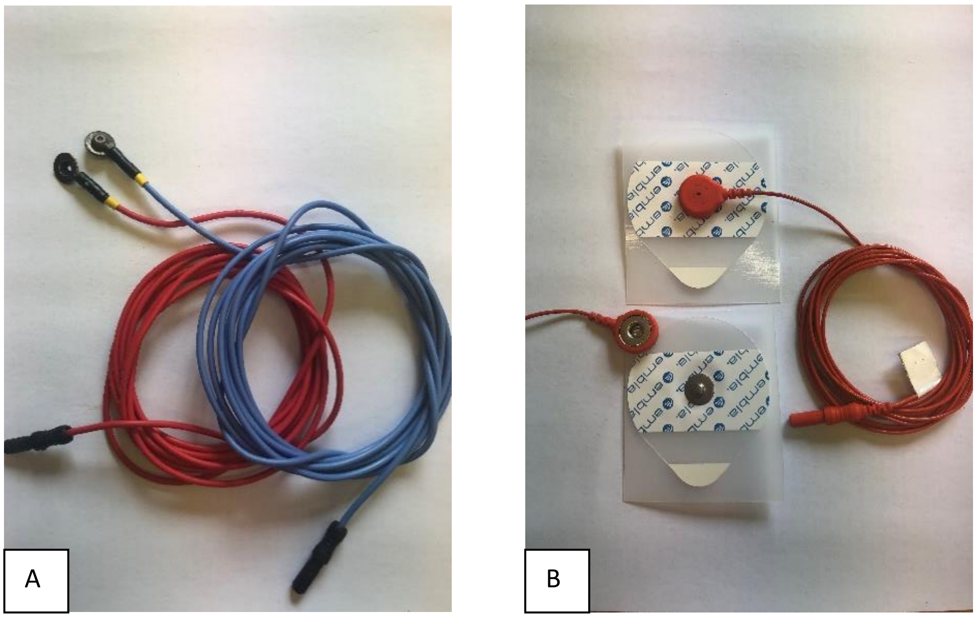


Figure 5. [A] Ag/AgCl cup electrodes, [B] pre-gelled adhesive snap ECG electrodes and leads

Electrode Removal

The electrodes could be removed by gently pulling on the electrode lead wire (Cup electrodes) or on the adhesive tab (ECG electrodes) and usually pulled away easily with no observed discomfort to the cow. If they could not be removed with gentle pulling, the glue was softened with aqueous cream or removed with acetone. Any glue remaining on the skin was removed with cream or acetone.

Device Housing

After attachment, electrode lead wires were bundled in a cable sleeve along the neck and plugged into the Embletta MPR PG and ST+ Proxy PSG recording device (Embla Systems, Kanata, Canada). The device was then placed in a protective plastic box lined with foam (Figure 6), placed in a soft sided zippered pouch (modified lunchbox), and attached to an elastic surcingle girth around the cow (Figure 7c). The modified lycra horse hood (Figure 7a) was placed over the top of the electrodes and wires to prevent them from being scratched off or snagged on features in the test pen environment. The horse hood was modified to better fit the cows' ears, as well as to create an access flap secured with Velcro so that electrodes on the forehead could be accessed without the need to remove the entire hood. Due to warmer than average temperatures during recording in the summer months in Scotland, and concern for cow comfort in the heat, a modified head-only version of the hood was created (Figure 7b). This 'summer hood' offered protection for the electrodes on the head, and bundling of electrodes on the neck, however, did not cover the neck and forequarters and may have been cooler for the cows during this time. For outdoor recording in New Zealand, the hoods were sprayed with a weatherproofing spray (Scotchgard Heavy Duty Water Shield, 3M New Zealand Ltd, Auckland) to protect the electrodes from wet weather conditions. A plastic shower cap was used as a cover over the top of the device housing pouch, to further protect the recording devices from the weather (Figure 7c).

Figure 6. Image of the Embletta MPR PG with ST+ proxy and attached snap electrode leads in the foam padded sandwich box.

Figure 7. [A] Cow in New Zealand wearing a modified Lycra hood [B] A cow in Scotland wearing a modified 'summer' hood with electrode wires bundled along the neck to the device housing attached to the girth, [C] A cow in New Zealand wearing the full hood and with a waterproof plastic cover over the device housing pouch.

Recording protocol

When all the electrodes had been attached, and the leads were plugged into the Embletta MPR PG recording device, it was essential to check that impedance (electrode-skin contact) was within an acceptable range (<14kΩ) before recording commenced. Impedance issues could be caused by numerous factors including hair regrowth, insufficient skin cleaning or preparation or issues with the device. Electrodes with impedance out of acceptable ranges were removed, these issues were addressed, electrodes were re-placed, and impedance was re-checked until an acceptable impedance value was achieved.

EEG, EOG and EMG were recorded using the Embletta MPR PG and ST+ Proxy (Natus Medical, Kanata, Canada). The device was programmed to record 4 channels of EEG, 2 channels of EOG, 2 channels of EMG, a reference electrode and ground electrode. Due to the number of electrode channels attached the maximum recording time was 10 hours due to the memory limitations of the device. Signals were sampled at 500Hz and the device was programmed to complete automatic calibration and impedance tests at the start of each recording.

Recordings ran from approximately 7am-5pm and 7pm-5am, however some variation in the start/end times of recordings occurred due to difficulties in device attachment/impedance testing, electrodes becoming detached mid-recording or other extraneous factors. Recording in the Scottish cows was occasionally disrupted when cows began showing oestrus behaviours. In these cases, the recording session was postponed, and the cow was returned to the group as oestrus behaviour would likely be disruptive to sleep patterns, and the cow could be more difficult to work with during this time.

Recording Data Quality

Each recording was checked for quality after data collection and download was complete.

Good quality PSG occurred when at least one EEG, EOG and EMG electrode remained attached for the entire recording time (or for a long period of time) and that impedance of these electrodes were within an acceptable range (<14kΩ). Impedance checks were automatically recorded on each trace at the start of the recording.

Signal Processing

At least one EEG, one EOG and one EMG trace were selected for analysis from each recording. High pass and low pass filters were applied to each trace to remove some of the noise due to attenuation of the signal, skin and muscle activity and noise in the environment and the gain was adjusted to adjust focus on the important features of the traces (Table 2.). A 50 Hz notch filter was applied to all traces to remove artefacts from the electrical mains frequency in Scotland and New Zealand.

Table 2. Filters and gain of cow EEG, EOG and EMG traces for PSG scoring

Trace	Low Pass	High Pass	Notch Filter	Gain
EEG	30 Hz	0.3 Hz	50 Hz	50µV/20µV
EOG	20Hz	0.15 Hz	50 Hz	500 µV
EMG	-	10Hz	50 Hz	50 µV

Detailed scoring protocol

As is standard in human sleep scoring, sleep stages were scored in 30 second epochs. Scoring was done by the author (LH) after completing training in *Practical Polysomnography*, a course approved by the Federation of the Royal Colleges of Physicians of the United Kingdom for full polysomnography including scoring sleep stages, arousals, and respiratory events according to AASM standards. The development of sleep staging criteria was based on methods described by previous publications regarding sleep in cows, (Hänninen et al., 2008; Ternman et al., 2012; Toutain & Ruckebusch, 1973) and on AASM guidelines (Iber & American Academy of Sleep

Medicine, 2007) and included observations of the overall wave patterns, FFT power spectrum of EEG traces, presence of eye movements and amplitude of the EMG traces.

Traces were first scored with the scorer blind to the behaviour or any other details from the cow during the recording. The scoring was then re-checked while simultaneously watching video recordings from 4 angles of the pen of the cow matched to the time of the traces. This allowed for the identification of artefacts such as ear flicks or movements that could appear to be blinks or other PSG features. Traces were then checked over a third time prior to completion to ensure all potential disrupting factors had been identified.

Scoring Criteria:

This scoring criteria describes 6 stages from wakefulness (W), N1, N2, N3, REM and rumination using EEG, EOG, EMG as well as the Fast Fourier Transform (FFT) power spectrum to measure frequency content and sleep power bands calculated from one EEG trace. PSG traces can appear visually different between cows and a certain amount of familiarization with the traces was required to adapt to each scoring session. Scoring was not based on the visual appearance of only one trace, but rather from a combined interpretation of all aspects. Epochs were scored based on the sleep stage that makes up >50% of the epoch. If the epoch was estimated to contain equal amounts of each sleep stage, it was scored based on the stage of the epoch immediately pre-ceding it. Epochs containing short artefacts that did not obscure the entire time period could be scored, but epochs with multiple artefacts for example from moving or eating (Table 3) were marked as unknown if the sleep stage could not be reliably determined.

Awake:

The awake state consists of low amplitude mixed frequency waves and appears as a thick and dark trace, this can be seen by the wide range of values in the EMG trace shown in Figure 8. There are often many artefacts from movement/eating etc during this stage. The FFT power spectra of EEG traces in the 10-30 Hz range is usually well above 300µV, however, can be at the 300-400 µV range while the cow is awake. There is a lot of activity in the EMG trace, which usually has high amplitude and frequency throughout with many artefacts from movement and muscle twitches. Frequent spikes are seen on the EOG trace from blinking. Figure 8 (a) indicates an epoch scored as awake during which the cow was standing and somewhat more active than the epoch in figure 8 (B) indicating an awake epoch of a cow who was lying down.

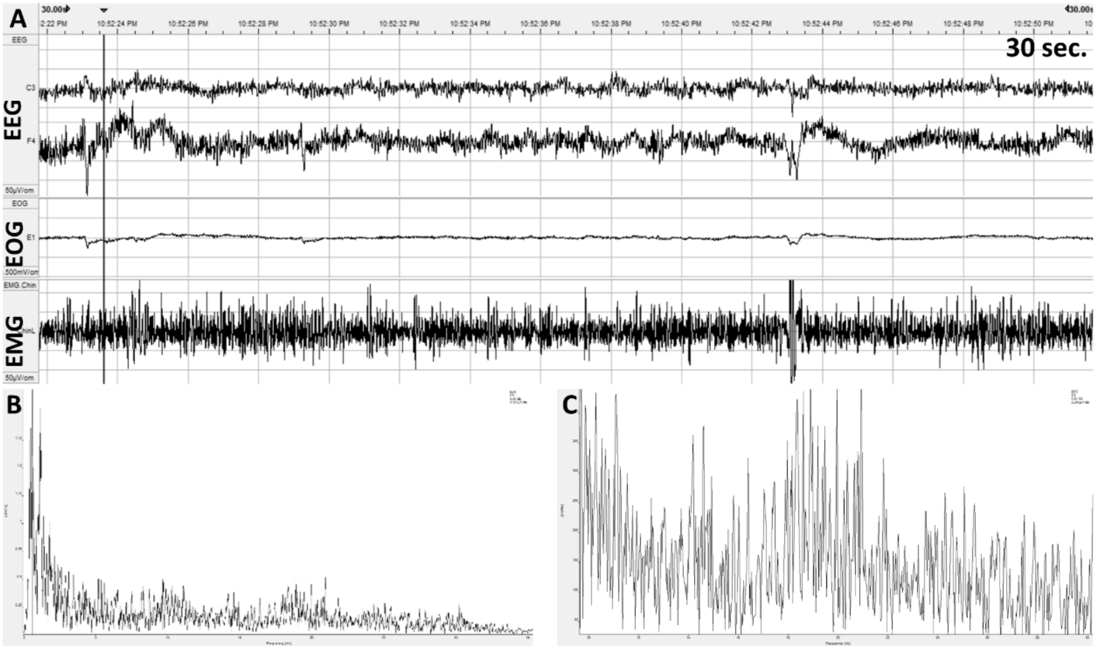


Figure 8. Screenshot from RemLogic software indicating an awake epoch; (A) Filtered PSG traces, (B) FFT power spectrum from 0-35 Hz, (C) FFT power spectrum for only the 10-30 Hz range.

N1:

Visually, the EEG in N1 appears to be similar to awake, however the trace is lower in amplitude and frequency and therefore the density of the trace appears to be lighter (See Figure 9 below). In this stage there are minimal artefacts from movement and activity. The FFT power spectra of the EEG trace in 10-30 Hz range is at or below 400µv. The neck EMG continues to show high amplitude and frequency and occasional activity on the EOG trace is seen (Figure 9). This stage was also very rarely found to occur while the cow was standing.

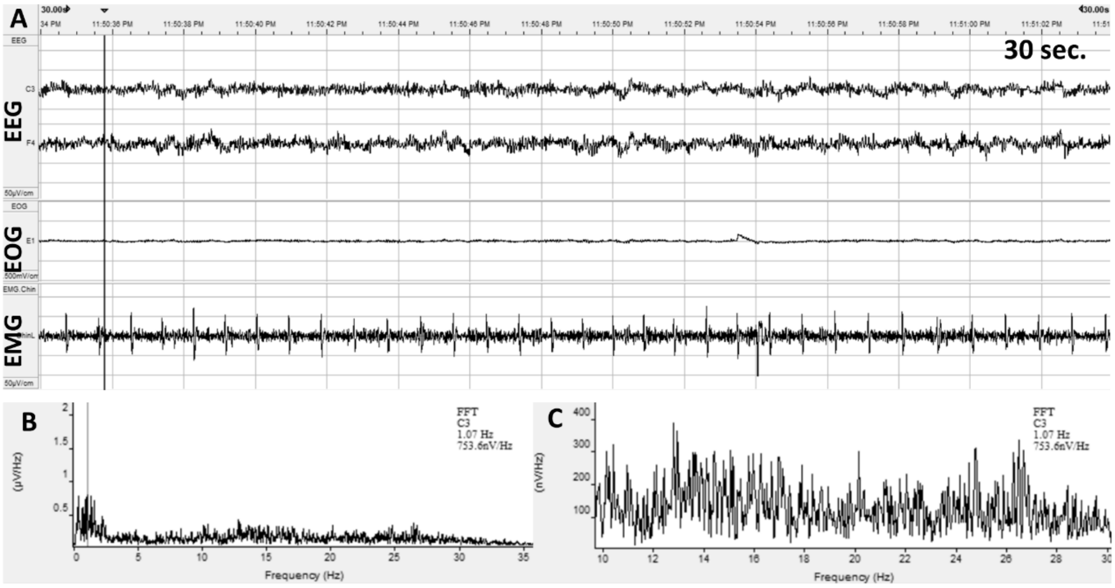


Figure 9. Screenshot from RemLogic software indicating an N1 epoch; (A) Filtered PSG traces, (B) FFT power spectrum from 0-35 Hz, (C) FFT power spectrum for only the 10-30 Hz range.

N2:

In this stage, low amplitude high frequency waves mixed in with higher amplitude low frequency wave patterns are observed on the EEG trace. The presence of features such as occasional spikes that resemble human k-complexes begin to occur, and incidences of small segments of high frequency waves that resemble human sleep-spindles can also be found. The FFT power spectra of the EEG trace in the 10-30 Hz range is well below 300µV. Activity on the EMG trace tends to remain high, with high frequency activity observed (Figure 10). Very few eye movements are observed on the EOG trace and movement artefacts are infrequent (Figure 10). Sleep spindles are observed and consist of a small burst of fast activity lasting <1second (Figure 11).

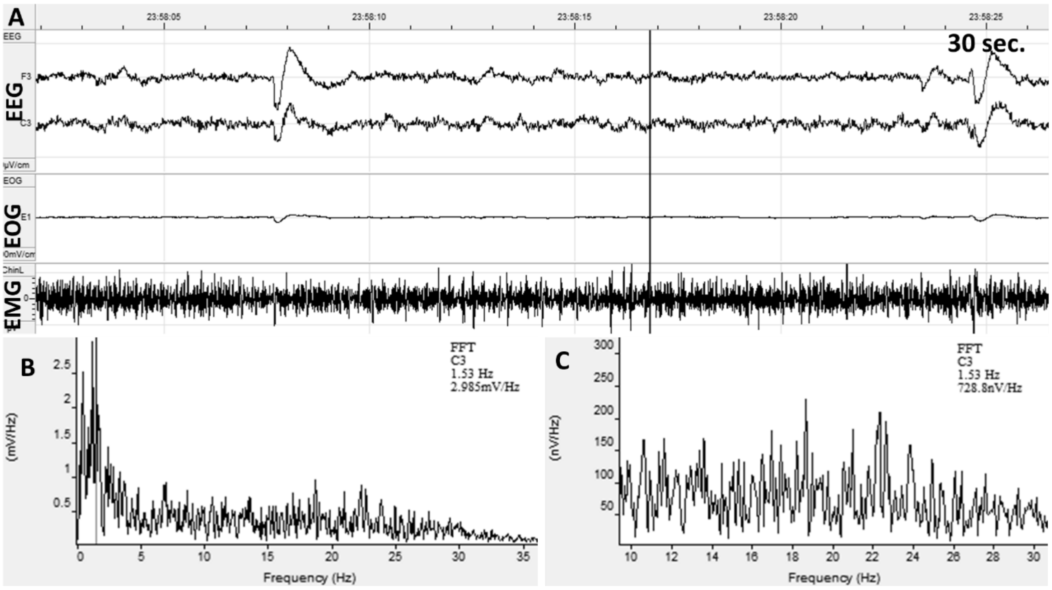


Figure 10. Screenshot from RemLogic software indicating an N2 epoch; (A) Filtered PSG traces, (B) FFT power spectrum from 0-35 Hz, (C) FFT power spectrum for only the 10-30 Hz range.

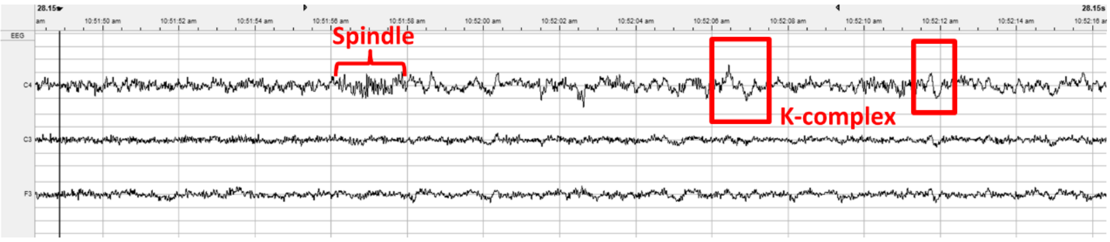


Figure 11. Screenshot from RemLogic software of the EEG traces from an N2 epoch containing probable sleep spindles and k-complexes (indicated in red).

N3:

In N3 stage, lower frequency and high amplitude waves are usually seen on the EEG trace. Compared to human N3 stages, which are often observed as high amplitude slow waves, the amplitude of the cow EEG traces during N3 may not be overly different from other N2 or REM stages. The slow wave pattern is seen most of the time, however N3 stage can also be identified as being when amplitude and frequency are both low. The FFT power spectra of the EEG trace in the 10-30 Hz range is usually well below 200µV (Figure 12). Neck EMG activity is usually low, however may not be drastically different from N2, and a heartbeat artefact may be seen on the trace as repetitive spikes. No eye movements are observed on the EOG and no movement artefacts occur.

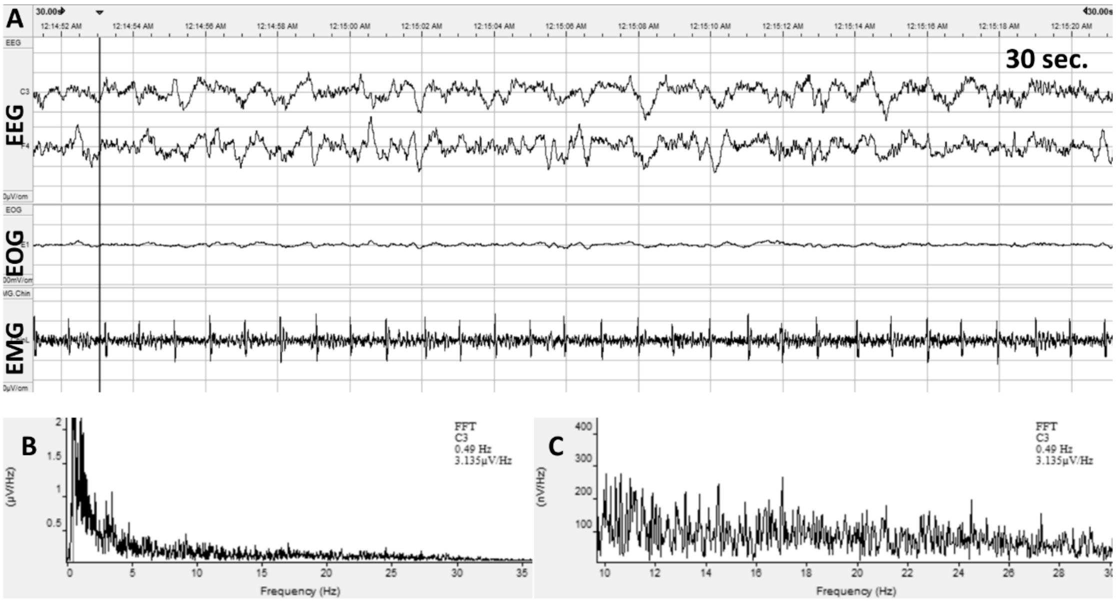


Figure 12. Screenshot from RemLogic software indicating an N3 epoch; (A) Filtered PSG traces, (B) FFT power spectrum from 0-35 Hz, (C) FFT power spectrum for only the 10-30 Hz range.

REM:

The EEG trace during REM sleep is usually characterized by very low amplitude high frequency waves. The EEG trace does tend to resemble the awake EEG trace however is less dense in comparison. Many artefacts can occur in this stage such as phasic twitches, ear twitches and rapid eye movements resulting in spikes on all PSG traces. The FFT power spectra of the EEG in the 10-30 Hz range is usually very low (>200µv), however due to artefacts on the trace, could be higher, even above the 300µV range. The main defining characteristic of this stage is very low activity on the EMG trace and frequent spikes and movements on the EOG trace from rapid eye movements (Figure 13).

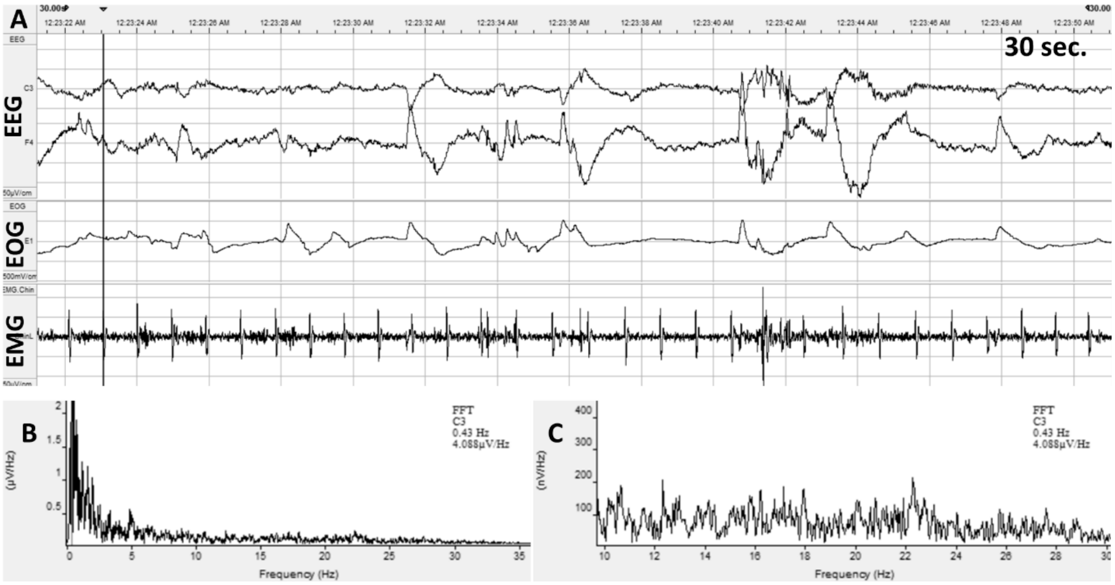


Figure 13. Screenshot from RemLogic software indicating a REM epoch; (A) Filtered PSG traces, (B) FFT power spectrum from 0-35 Hz, (C) FFT power spectrum for only the 10-30 Hz range.

Rumination:

The powerful chewing motion during rumination bouts causes a characteristic artefact that obscures signals from all PSG traces. This consists of rhythmic spikes and troughs with very high amplitude interspaced with bouts of swallowing lasting approximately 3-4 seconds (Figure 14).

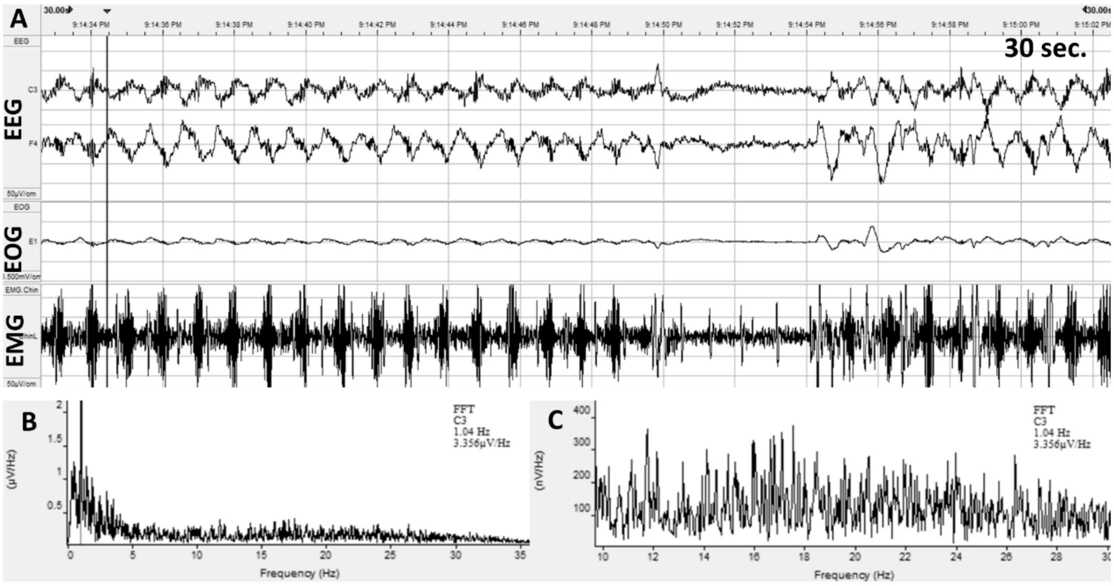
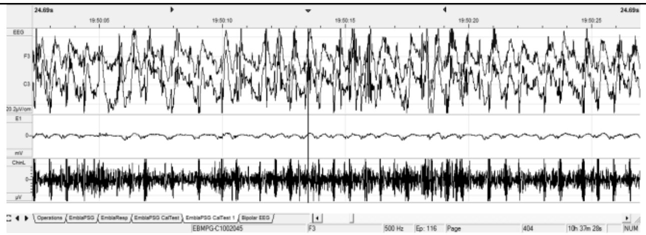
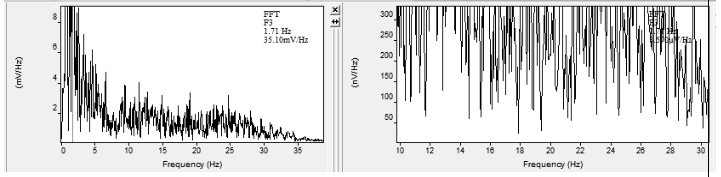
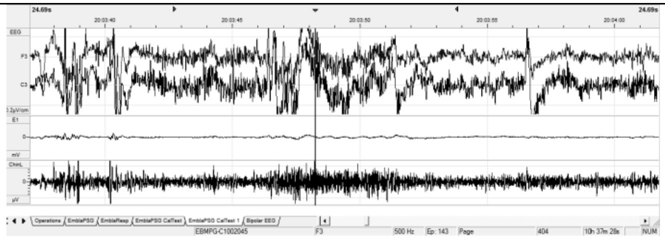
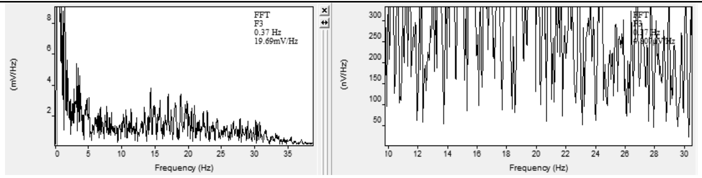
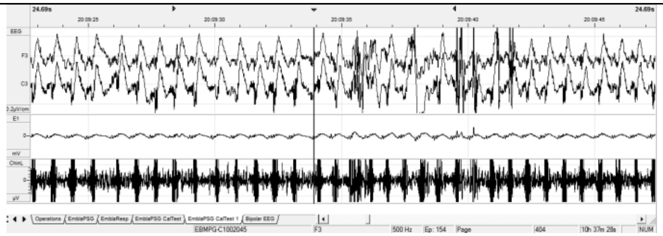
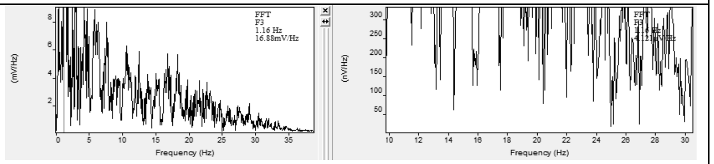


Figure 14. Screenshot from RemLogic software indicating a rumination epoch with swallowing; (A) Filtered PSG traces, (B) FFT power spectrum from 0-35 Hz, (C) FFT power spectrum for only the 10-30 Hz range.

Table 3. Table of commonly observed artefacts (Eating, movement and rumination) and images of the PSG traces (EEG, EOG and EMG), and FFT power spectrum from 0-35Hz range and 10-30 Hz range.

Artefact	**EEG, EOG, EMG**	**Power Spectrum**
Eating		
Movement		
Rumination		

Recorded Data Quantity and Quality

Two of the Scottish cows did not progress sufficiently through the training and habituation protocol and were removed from the study. In total, 590 hours of PSG recordings were collected from the 12 cows in both Scottish and New Zealand studies. From this, approximately 212 hours were rejected due to issues with signal quality or malfunction of other devices not discussed in this chapter. Approximately, 167 hours from the Scottish cows and 210 hours of data were scored and used for analysis from the New Zealand cows. Of the overall total data scored, 178.68 hours were recorded during the daytime and 199.02 hours were recorded at night. A further break down of the data recorded is included in appendix A.

Intra-scorer Reliability

Intra-rater reliability was computed using the `irr` package (Gamer et al., 2019) in R (version 4.0.5) (R Core Team, 2021) for the reliability of scoring sleep stages from the PSG. Overall agreement was 89.4% and a Cohen’s kappa value of k= 0.835 indicated a high level of agreement. Further investigation using Fleiss’ kappa to compute category-wise kappa values found significant differences in agreement between stages (Table 4). Very high and near-perfect level of agreement between scoring was found for awake (W) and rumination (RNT) epochs, a good level of agreement was found for N2, N3 and R stages, however a poor level of agreement was found for the reliability of scoring N1. N1 was mostly mistakenly scored as N2 in 39.1% of re-scored epochs or as REM in 28.3% of re-scored epochs (Table 5). As most scoring mistakes were made between N1 and N2 we decided to pool these stages to investigate the effect on overall agreement. Total percent agreement increased to 91.1% with a Cohen’s kappa of k= 0.86 indicating a high level of agreement. Agreement between the combined N1/N2 improved to k=0.691 (Table 4) with an agreement of 80.8% between original and re-scoring (Table 6) while kappa’s for other sleep stages remained unchanged.

Table 4. Table of categorical Fleiss's kappa values by sleep stage and level of agreement before and after combination of N1 & N2 stages

Sleep Stage	Kappa	Level of agreement	Sleep Stage	Kappa combining N1/N2	Level of agreement
W	0.880	Strong	W	0.880	Strong
N1	0.295	Minimal	Light NREM	0.691	Moderate
N2	0.681	Moderate			
N3	0.755	Moderate	N3	0.755	Moderate
REM	0.697	Moderate	REM	0.697	Moderate
RNT	0.973	Almost Perfect	RNT	0.973	Almost Perfect
UN	0.267	Minimal	UN	0.267	Minimal

Table 5. Percentage of agreement between first scoring and re-scoring by sleep stage

	W	N1	N2	N3	R	RNT	U
W	86.1%	6.4%	0.3%	0.0%	1.3%	0.3%	5.6%
N1	0.0%	32.6%	39.1%	0.0%	28.3%	0.0%	0.0%
N2	0.0%	3.4%	84.5%	6.9%	5.2%	0.0%	0.0%
N3	2.3%	0.0%	30.2%	67.4%	0.0%	0.0%	0.0%
R	10.4%	0.0%	2.1%	0.0%	87.5%	0.0%	0.0%
RNT	0.3%	1.0%	0.0%	0.0%	1.1%	97.6%	0.0%
U	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%

Table 6. Percentage of agreement between first scoring and re-scoring by sleep stage after combining N1 & N2 sleep stages

	W	N1/2	N3	REM	RNT	UN
W	86.1%	6.7%	0.0%	1.3%	0.3%	5.6%
N1/2	0.0%	80.8%	3.8%	15.4%	0.0%	0.0%
N3	2.3%	30.2%	67.4%	0.0%	0.0%	0.0%
REM	10.4%	2.1%	0.0%	87.5%	0.0%	0.0%
RNT	0.3%	1.0%	0.0%	1.1%	97.6%	0.0%
UN	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%

Discussion:

We developed and described a 5-stage visual scoring system for sleep for dairy cows, based on human AASM guidelines and previous published work with dairy cows, and have shown strong intra-observer reliability in the range of inter-observer observations from human sleep scoring studies. However, we have identified that there are differences in the reliability between categories of sleep stages. Using this scoring system, we showed very good ability to identify N2, R, RNT and a reasonably good ability at identifying N3, however most disagreements in the intra-rater reliability were between N1 and N2 stages in cows. This is similar to results found for intra-observer reliability in a human study where a registered PSG scoring technician with 33 years of experience also had more disagreements between N1 and N2 sleep (Suzuki et al., 2019).

This scoring system is the first to score lighter stages of NREM sleep in dairy cows. Previous criteria for scoring sleep in dairy cows described REM sleep, Slow Wave Sleep and ‘drowsing’. Ruckebusch (1972) described drowsing as a stage of stable wakefulness, indicating that he believed the cows to be awake but inactive during this time. More recent work in cows that have also scored drowsing according to the criteria described by Ruckebusch, and noted the occurrence of sleep spindles and k-complexed during this stage (Ternman et al., 2012). Thus, stages previously described as drowsing might likely be a combination of N1 and N2 light sleep stages. The description of the 5 stages of sleep in this study are based solely on their visual similarity to well described human sleep stages. Essentially, human sleep has been differentiated into these stages as a way to categorize different brain patterns as well as depths of sleep. Sleep depth can be determined from auditory arousal thresholds (Busby et al., 1994; Pilon et al., 2012) and generally, it takes less auditory stimulation to wake someone from light sleep than from a deep sleep. In future, verification of the classification of sleep stages in dairy cows could be accomplished by investigating the auditory arousal thresholds compared to PSG.

A considerable amount of data was not usable due to poor signal quality, electrodes falling off, and other issues with the adaptation of these devices, that are intended for humans, for dairy farm conditions. While PSG is an excellent way of accurately identifying sleep stages of dairy cows it is not an ideal way to study sleep in a large number of cows or to use as a tool to assess welfare of cows. Accurate, more accessible and easily used alternative methods would be beneficial for future studies of sleep-in dairy cows.

Conclusion

PSG is challenging and onerous to use with dairy cows, and even after developing substantial practical experience with PSG and improving efficiency in the methodology, we continued to experience a large amount of data loss and poor data quality. This data loss was likely due to the cows' behaviour and the device being il-suited for use outside of clinical settings. Being able to identify sleep in cows accurately is the first step in being able to study sleep, and how changes to sleep patterns and architecture correspond with cow welfare. We developed a 5-stage scoring system for cow sleep, based on modern human scoring guidelines as well as limited previous cow work. Overall intra-observer agreement was strong, however reliability for the identification of light N1 sleep was minimal. Combining N1 with N2 into "light sleep" improved agreement to a moderate level. N1 can be reliably distinguished from awake, indicating that it is likely to be a distinct stage of sleep, but more work is needed to improve definitions to distinguish N1 from N2 in the future. Identifying sleep stages accurately from PSG is essential for the investigation of how sleep architecture affects sleep quality and thus cow welfare and is also pivotal for the investigation of possible methods of recording sleep in cows as an accurate alternative to PSG. The ensuing chapters in this thesis utilize the described methods and scoring system to identify sleep stages using PSG as a baseline or ground truth with which to examine other non-invasive proxy indicators for sleep in dairy cows.

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CHAPTER THREE

Lying posture does not accurately indicate sleep stage in dairy cows

Preface

The body of this chapter comprises a paper that has, since submission of this thesis, been published in Applied Animal Behaviour Science (AABS) journal. The structure of this chapter is therefore in the AABS style, however, has been modified to fit with the format of this thesis and may differ from the version available online.

Hunter, Laura B., O'Connor, C., Haskell, M.J., Langford, F.M., Webster, J.R., Stafford, K.J., 2021. Lying posture does not accurately indicate sleep stage in dairy cows. Appl. Anim. Behav. Sci. 242, 105427. https://doi.org/10.1016/j.applanim.2021.105427

Following on from the development of the 5-stage scoring system for cow sleep described in the previous chapter, the aim of this chapter was to begin to investigate alternative proxies for PSG to assess detailed sleep stages in dairy cows. Behavioural observations can be conducted with minimal to no disturbance to cows and would be the most straightforward and least invasive method for the cows and therefore better for their welfare. However, as mentioned in chapter one, there is some uncertainty regarding the reliable use of lying postures to estimate sleep stages in cows. Previous studies have indicated that lying postures can be used to identify REM and NREM sleep in calves, however the same postures were not able to be used to identify these two stages in adult cows. Therefore, specifically, the aim of this chapter was to determine if more detailed head positions and lying posture could be used to identify the 5-stages of sleep in cows.

Hänninen, L., Mäkelä, J.P., Rushen, J., de Passillé, A.M., Saloniemi, H., 2008. Assessing sleep state in calves through electrophysiological and behavioural recordings: A preliminary study. Appl. Anim. Behav. Sci. 111, 235–250. https://doi.org/10.1016/j.applanim.2007.06.009

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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

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Lying posture does not accurately indicate sleep stage in dairy cows

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Key Words: Dairy cows, Sleep, Lying behaviour, Posture, Polysomnography

Abstract

Quality sleep is important for physical health and welfare in animals. However, we know little about dairy cow sleep, and how much they need. Practical techniques are needed to monitor sleep in cows to determine how different management practices affect their sleep and their welfare. It is impractical to use 'gold standard' electrophysiological - polysomnography (PSG) to identify sleep in cows. Previous work suggests lying postures are useful to identify sleep stages in calves, but the reliability of lying behaviour to identify these sleep stages in adult cows is uncertain. We compared the lying postures of adult dairy cows (deep bedded on straw or in a pasture) with PSG, to determine if lying postures could be used to accurately identify Rapid eye movement (REM) and the different stages of Non-REM sleep. Lying in the typical "sleep" posture with the head turned and resting on the flank identified approximately 70% of REM sleep in outdoor managed cows but was less accurate in indoor housed cows that showed REM sleep in numerous postures. Lying with the head still and low did not identify stages of Non-REM sleep in either group. Using sleep postures to estimate total sleep would be an over estimation of REM sleep, but also an underestimation of total sleep as it ignores most NREM sleep. Lying postures are not useful indicators of sleep stages in dairy cows and additional research is required to identify efficacious alternative techniques.

Introduction

Sleep is an essential physiological function for all animals and plays an important part in physical and mental health (Faraut et al., 2012). Little is known about the sleep needs of dairy cattle or its impact on cow health, productivity, and welfare. Sleep can be disrupted by stress or environmental factors such as light (Raap et al., 2016) and comfort (Troynikov et al., 2018). Therefore, the amount of sleep and changes to sleep patterns could be used as a tool for animal welfare assessment (Langford & Cockram, 2010). It is challenging, however, to identify when a cow is sleeping and the different stages of sleep.

Sleep in humans and other animals is typically sub-divided into several stages from light to deep sleep. These different stages allow different functions such as memory processing in deep rapid eye movement (REM) sleep (Boyce et al., 2017; Siegel, 1995), and restorative functions during Non-REM (NREM) sleep (Xie et al., 2013). In humans and some animals, NREM sleep can be further divided into light stages (N1 and N2) and deeper slow wave sleep (N3) (Goonawardena et al., 2019). Much of the sleep literature in humans and animals focusses on REM sleep, however, NREM sleep and particularly lighter stages (N1 & N2) makes up the majority of human sleep time (McCarley, 2007) and are also likely to be important features of sleep for other animals. Therefore, it is important to record light sleep stages when investigating effects of housing and management on sleep in cattle.

The recognized gold standard for assessing sleep in animals is polysomnography (PSG). This involves recording electrophysiological signals (traces) of the brain (electroencephalogram- EEG), eye movement (electrooculogram- EOG) and muscle activity (electromyogram- EMG) which are scored into sleep stages by visual analysis of the characteristic patterns of these traces. PSG recording systems require many electrodes, wires and fragile and expensive signal amplification/recording devices that are usually made for use on humans. While they can be used with large animals, they are not an ideal tool for the acquisition of sleep data in cows managed under standard farm conditions which may be humid and dirty, or used in a large-scale scientific study with other animals that may disrupt the devices.

Dairy cows sleep lying down (Ruckebusch et al., 1970) and can adopt several different postures while lying which could be useful to identify different sleep stages. One of the main characteristics of REM sleep is a reduction in muscle tone on the EMG trace, which reflects relaxation of the major muscle groups. In cows, this may necessitate relaxation and resting of the head. Recent studies have described the 'sleep posture' as being when a cow is lying sternally with her head rested on the flank and have used this to estimate total sleep time in cows (Fukasawa et al., 2020). Scoring sleep in this manner however is likely to be a better estimate for REM sleep and an underestimation of total sleep time, as time in NREM sleep will not be included. In calves, lying with the head resting on the ground or on the flank, identified REM sleep and lying with the head held up identified NREM sleep as determined from PSG (Hänninen et al., 2008). Although, in indoor housed adult cows, these same postures could not be used as reliably to identify the same sleep stages (Ternman et al., 2014), perhaps due to changes in sleep behaviour with age.

These previous studies have only scored deep NREM sleep (N3) and have not differentiated between light and deep NREM sleep stages (N1 and N2 vs N3). In deep NREM sleep, a reduction in muscle tone is also seen on EMG traces as it is for REM sleep (Hänninen et al., 2008). This reduction is not usually as great as the atonia associated with REM sleep and therefore could result in a lower head posture but may not necessitate head resting. The position of the head could therefore potentially be useful to identify differences between light and deep sleep stages as deeper NREM sleep may be associated with the head held lower or in a more 'relaxed' position.

To our knowledge, all previous studies of sleep in dairy cows using PSG have been conducted in indoor-housed environments. Pastoral dairy systems add additional challenges as animals are outdoor ranging and may be more active, potentially affecting device attachment, signal quality and increasing risks of weather damage to the equipment used in PSG. It is also unknown if the environment and conditions including the space available for lying and the lying surface, influences posture during sleep.

The aims of this study were to investigate if a more detailed behavioural assessment of lying postures and neck position is useful to accurately differentiate between different stages of light to deep sleep and wakefulness in adult dairy cows, both in an indoor housed environment in Scotland (UK) and outdoors on pasture in New Zealand (NZ). Specifically, we aimed to determine if lying with the head resting can be used to predict REM sleep, if lying with the head lifted but still and up would be an indicator of lighter NREM sleep (N1 and N2) and if lying with the head still and low could be used to identify deep NREM (N3) sleep.

Methods

This study was designed in accordance with relevant animal welfare guidance and regulations. Ethics approval was obtained prior to any procedures involving animals. In the UK, Ethics approval was obtained from SRUC animal ethics committee (ED AE 03-2018) and was carried out under Home Office Licence (P204B097E). In NZ animal ethics approval was obtained from Ruakura animal ethics committee (NZ AE 14708).

Housed Animals (UK)

Six non-pregnant Holstein-Friesian cows, average age 3.8±0.6 years and known to have a calm nature were selected from the herd at the SRUC Dairy Research Centre, Acrehead Farm (Dumfries, Scotland UK). All cows were in good health and were due to be retired from the herd due to poor reproductive performance. They had been dried off according to routine farm practice prior to the study. Halter training and habituation to the procedures and materials used in the study were conducted prior to recording using positive reinforcement techniques with concentrate pellets as reinforcers. Following the training and habituation period, recording was conducted in a 5x5m ‘test pen’ directly adjacent to the larger group pen. A 1-2m buffer zone was created between the pens to discourage group mates from interfering with the recording devices (Figure 1). Visual and auditory contact with group mates, as well as the rest of the herd was always possible. Cows had *ad libitum* access to silage and fresh water in both test and group pens. All device attachment and maintenance were conducted with the cow in the test pen.

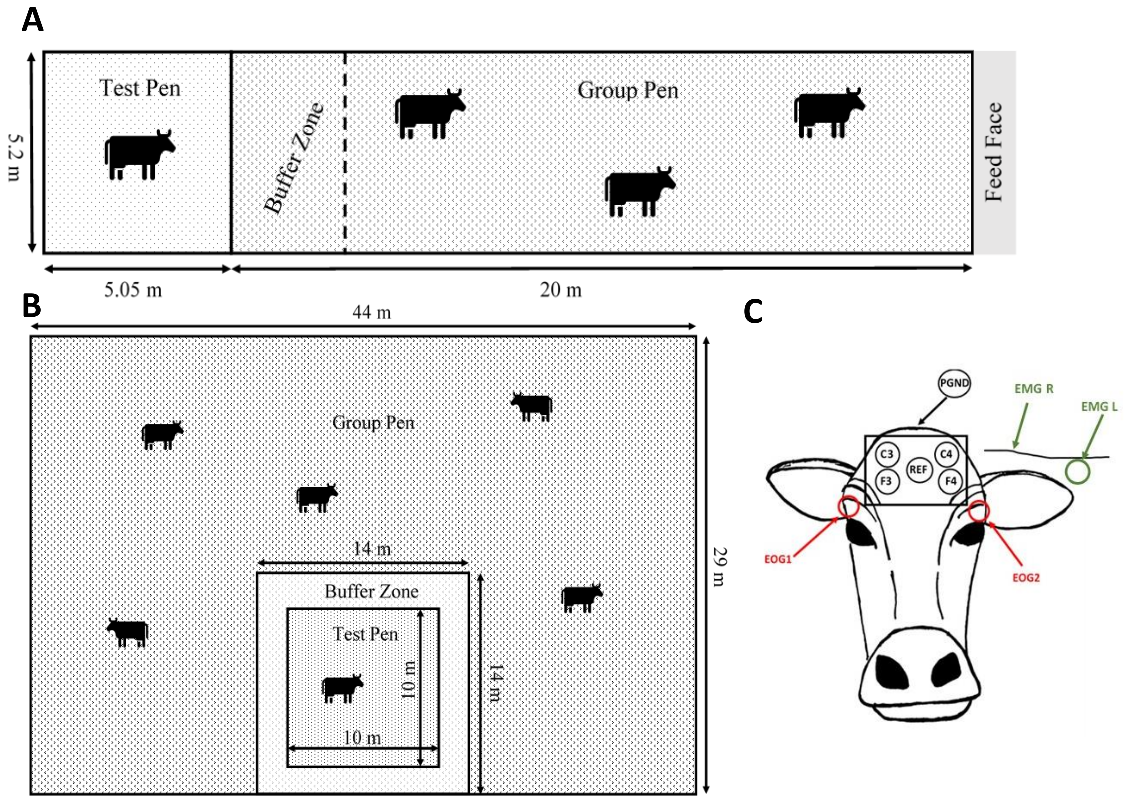

Figure 1. Diagrams of group and test pen and PSG electrode locations**.** (A) Depicts the indoor pen set up in the UK and (B) depicts the NZ outdoor pasture pen set up created with electric fencing within a larger paddock. During recordings, the test cow was moved into the test pen, when not recording, the cow was moved back into to the group pen. Diagram (C) depicts the electrode locations on the test cow's head and neck for PSG recording, including EEG (C3, C4, F3 and F4), two EOG electrodes, two EMG electrodes on either side of the neck as well as a reference electrode (REF) and a patient grounding electrode (PGND) placed behind the poll on the top of the head.

Pasture Animals (NZ)

Six three-year-old mid to late pregnant dry Kiwi-cross cows were selected for their quiet and approachable nature at Dairy NZ Lye Farm (Newstead, NZ). The cows were halter trained and habituated to being touched and handled and to experience the equipment used in the study with positive reinforcement techniques using grass silage as a reinforcer. Recording was conducted outdoors in a typical NZ paddock environment. A portable 10x10m test pen was constructed using electric fencing tape and standards. The fence was not electrified so as not to interfere with the electrophysiological recording device. A 2-meter buffer zone around the test pen was created using live electric fencing tape to separate the test cow from the other 5 groupmates in the paddock surrounding the test pen (Figure 1). Visual and auditory contact between test cow and groupmates was always possible. All cows had access to fresh water and pasture, and supplementary silage was provided several times daily. The test pen and group pen were rotated to a new area in the paddock every 1-1.5 weeks to prevent ground conditions from becoming wet and dirty and to provide the cows with fresh pasture to graze. The cows were moved into a sampling race for device attachment, and daily device maintenance was conducted with the haltered cow in the test pen.

Cow Preparation and Device attachment

The hair at electrode attachment sites was clipped and the skin was cleaned thoroughly to improve impedance. Adhesive ECG electrodes (Natus Neurology, Kanata, Canada) were placed as indicated (Figure 1c) using a small amount of super glue (Loctite 454 or Loctite gel control, Henkel Corp., Dublin, Ireland) around the adhesive edge to ensure a secure attachment. Electrode leads were clipped on the appropriate electrode, bundled along the neck and plugged into the Embletta MPR PG with ST+ proxy polysomnography recording device (Natus Neurology, Kanata, Canada), housed in a protective pouch attached to the cow with an elasticated surcingle girth. A Lycra horse hood (UK: Horse Health Wessex, Woodington, UK. NZ: Caribu AU, Truganina, Australia) modified to fit a cow was placed over the head to hold the leads closely to the body to avoid entanglement and protect electrodes from being rubbed off. In the outdoor NZ study this hood was coated in breathable weatherproofing spray (Scotchgard Heavy Duty Water Shield, 3M New Zealand Ltd, Auckland, NZ) and a water-proofing plastic cover was placed over the device housing pouch to further protect the recording materials from the elements.

Recording and Sleep Scoring

RemLogic 3.4.3 software (Embla Systems, Kanata, Canada) was used to set up, download and score PSG data. The Embletta device was programmed to record four channels of EEG, two channels of EOG, two channels of EMG. A reference electrode and grounding electrode were also attached (Fig 1c). Immediately following attachment, each electrode's impedance was checked to be within an acceptable rage (1-14kHz). If it was not, the electrode was removed, the skin cleaned, and the electrode was attached and re-checked until impedance was within range. Data were sampled at 500Hz and due to memory and battery limitations of the Embletta device, recordings were restricted to 10 hours in length and ran from approximately 7am-5pm and 7pm-5am.

At the end of the 10-hour recording period, data were downloaded from the device and checked for quality. "Good" recordings occurred when all impedance was within range (>14Ω) and at least 2 EEG, 1 EMG and 1 EOG electrodes remained attached for the entire recording and could be used for scoring. 'Poor Quality' recordings occurred when impedance had deteriorated between attachment and recording, or the reference/ground electrodes had become detached, battery malfunctions leading to data loss, extreme artefacts or poor impedance. These recordings were not scored.

Sleep stage scoring was conducted by as single scorer (LH) who had received training in human PSG scoring using American Association of Sleep Medicine (AASM) guidelines. Scoring criteria (Table 1) were developed based on AASM guidelines as well as criteria from previous publications of cow sleep with PSG (Hänninen et al., 2008; Ruckebusch et al., 1970; Takeuchi et al., 1998; Ternman et al., 2012). Unlike previous work, four stages of sleep from light N1, N2 to deep N3 and REM sleep stages as well as wakefulness and rumination were scored in standard 30 second epochs based on the vigilance state that made up >50% of the epoch. Intra-scorer reliability was calculated using the function within the RemLogic software, and percent agreement was 89.42%.

Table 1. Basic sleep stage definitions for awake, light N1, N2, deep N3 and REM sleep stages.

Sleep Stage	*Defining Features on the PSG traces*	
Awake	*EEG*	High frequency mixed amplitude, often higher amplitude, artefacts throughout trace.
	EOG	Many blinks and eye movements
	EMG	High frequency, high amplitude, many movement artefacts
N1	*EEG*	Can appear to be similar to awake- high frequency lower amplitude, no artefacts
	EOG	Very few if any eye movements
	EMG	High frequency, however lower amplitude than awake, few movement artefacts
N2	*EEG*	Lower frequency than n1, some lower frequency waves, occasional sleep spindles (short bursts of repetitive higher amplitude waves) and k-complexes (Sharp low frequency waves)
	EOG	No eye movements
	EMG	high frequency, lower amplitude than n1, no movement artefact
N3	*EEG*	Typically seen as low frequency high amplitude waves.
	EOG	No eye movements visible on trace
	EMG	Very low amplitude
REM	*EEG*	EEG- low amplitude mixed frequency waves, occasions very low amplitude.
	EOG	Occasional bursts of rapid eye movements.
	EMG	Low amplitude, occasional rapid muscle twitches, the heartbeat is often picked up as an artefact on the trace.

Behaviour Scoring

A Geovision monitoring system (UK) (Viewlog, GeoVision Inc., Taiwan) and Vivotek ND9541P H.265 NVR (Vivotek Inc., Taiwan) (NZ) recording system were used, with video cameras equipped with automatic infrared night vision mounted on each corner of the test pen to ensure views from all angles could be achieved.

Time stamps on the video recordings were synchronized with time stamps from the PSG recordings. Lying postures (Table 2) were scored instantaneously by a single observer from the video in 30 second increments to correspond with the start of the scored sleep epoch.

Intra-observer reliability for the lying posture data was calculated using the Cohen's kappa reliability function in the "psych" package in R (version 4.0.2). The kappa statistic was k= 0.95 indicating an almost perfect level of agreement (McHugh, 2012).

Epochs identified as rumination were removed from the analysis since sleep or wake stage could not be reliably determined from the PSG trace due to chewing artefacts on the traces. All standing epochs were also removed from the data set since cows sleep lying down.

Table 2. Behavioural ethogram for scoring lying postures in dairy cows, including head positions and photographs from surveillance videos.

Behaviour			*Description*	
Standing			Any activity when the cow's body is off the ground and the bodyweight is supported by the legs.	
Lying	*Head up and Still*	*Lying Head Up (UP)*	The cow is sternally recumbent, with the neck lifted and the poll of the head above the withers.	
		Lying Head Low (HL)	The cow is sternally recumbent with the neck held low but not resting on the ground and the poll of the head below the withers.	
	Head Rested	*Tucked (T)*	The cow is sternally recumbent with the neck turned backwards and the head resting on the flank or on the ground.	
		Flat-Out (FO)	The cow is laterally recumbent with the legs extended, head and neck resting on the ground.	
		Lying Head Front on Ground (HF)	The cow is sternally recumbent, with the neck low or on the ground and the head resting on the ground in front.	
	Moving (M)		The cow is in any of the above recumbent positions, but the head and/or neck are moving.	

Data analysis

Data from 2 of the 6 cows in the UK group were removed due to limited data as only one 'good' recording was available from these cows. Cross-tabulations of data were conducted, and positive and negative predictive value as well as sensitivity and specificity were calculated as described in equations 1-6. Sensitivity indicates the probability of the posture correctly identifying a target sleep stage out of all epochs scored as that sleep stage (true positives), and equally, not classifying the epoch as not being in the sleep stage, when in fact they are (false negative) (Trevethan, 2017). Specificity applies to epochs identified by the PSG as being not in the target sleep stage and defines the probability of correctly identifying epochs not in the target sleep stage (true negatives), and also avoiding false positives- classifying the epoch as the target sleep stage, when it isn't.

Positive predictive value (PPV) refers to the probability that of the epochs scored as positive (in this case in the target posture), that the target sleep stage was correctly identified (true positive), and equally, epochs are not classified as being in a particular sleep stage, when they in fact are not (False positive). Negative predictive value also applies to the epochs that were identified as not being in the target posture and defines the probability that when giving a negative result (i.e. not being in the posture) the probability that all cows are not in the sleep stage (true negative), and not categorizing some epochs as not being in the sleep stage when they actually are (False Negative).

$$Positive\ Predictive\ Value\ (PPV) = \frac{True\ Positive}{True\ Positive + False\ Positive}$$

$$Negative\ Predictive\ Value\ (NPV) = \frac{True\ Negative}{False\ Negative + True\ Negative}$$

$$Sensitivity = \frac{True\ Positive}{True\ Positive + False\ Negative}$$

$$Specificity = \frac{True\ Negative}{False\ Positive + True\ Negative}$$

Results

With two of the UK cows and all epochs of rumination removed from the data set, 91.06 hrs were obtained from 16 recordings from the UK cows (n=4) six of which occurred during the daytime and ten overnight. In the UK group, 57.2% of lying time (when not ruminating) were scored as being awake, and 42.8% as being asleep. The UK group spent 68.4% of the total recording time lying down, whereas the NZ group spent 37.8% of total recording time lying down. In the NZ group (n=6), 116.3 hrs of sleep/wake data were obtained from 24 recordings, half of which occurred in the daytime and half over night. 82.3% of total lying time when not ruminating were spent awake, and the cows were asleep for 17.7% of the non-ruminating lying time.

UK indoor-housed cow results

Table 3 displays the positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity for each of the postural predictions for sleep stages in the UK indoor-housed cohort. In this group, of epochs scored in the Tucked (T) posture, the PPV of correct identification of REM sleep epochs was 44.69%. Of epochs scored in other postures, the NPV was 98% of correctly identifying epochs not in REM sleep. The sensitivity of tucked lying posture to correctly identify REM sleep epochs was 62% and specificity was 93.23%.

Table 3. Positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of the Scottish indoor managed cows describing the use of specific lying postures ability to identify sleep stages.

Posture- Sleep Stage Identifier	*PPV*	*NPV*	*Sensitivity*	*Specificity*
Tucked Posture= REM	44.69%	98.34%	77.62%	93.23%
Resting Posture= REM	31.90%	99.57%	94.69%	85.76%
Head Low= N3	6.13%	94.97%	58.36%	46.79%
Head UP= N1/2	18.66%	68.14%	14.75%	73.94%
Head Low= NREM	34.80%	65.92%	54.02%	46.78%

Including all postures when the head was resting on the flank or on the ground (T, FO, HF) did not improve positive predictive value for REM sleep in the UK group, which declined to 31.9%. NPV for this combination of postures was high. When not in a head resting posture, there was a 99.57% chance that a cow was not in REM sleep. Of epochs scored in REM sleep, sensitivity of head resting postures to correctly identify REM sleep was 94.69% and specificity (probability that not being in a head resting posture correctly identified epochs not in REM sleep) was 85.75%. As seen in Figure 2a, all sleep stages were observed in the FO and HF postures.

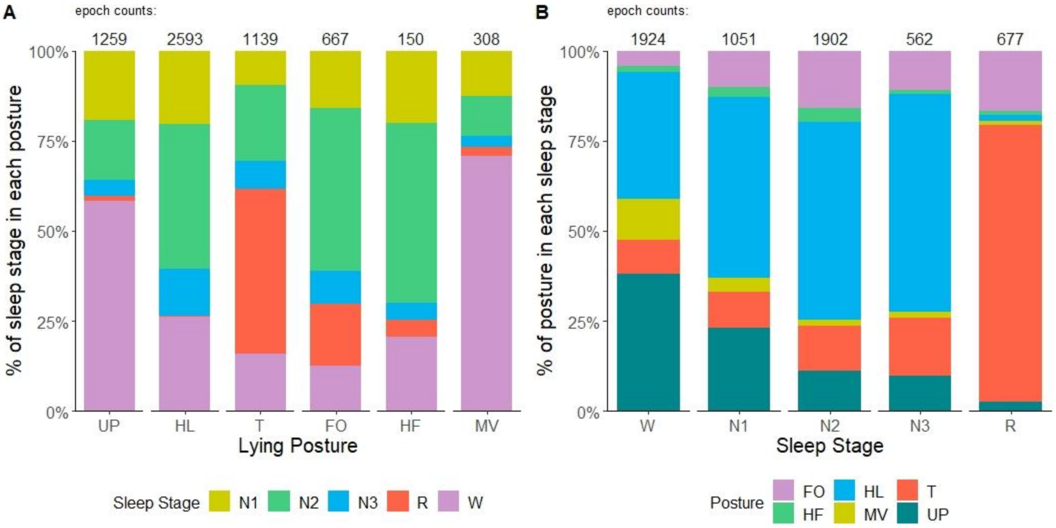

Figure 2 Proportions of sleep stage epochs that occurred in each lying posture in the Scottish indoor-housed group (n= 4 cows) with total epoch counts of each posture above each bar (a), and proportions of lying posture epochs that occurred in each sleep stage in the indoor housed group with total epoch counts of each sleep stage above each bar (b). Figure produced in R version 4.0.5 using ggplot2 package (https://cran.rproject.org/web/packages/ggplot2/index.html)

Lying with the head held immobile and below the shoulder (HL) was not a good predictor of N3 sleep. Of epochs scored in the HL posture, the probability of correctly identifying N3 sleep (PPV) was only 6.13% and NPV was 94.97%. Of epochs scored as N3 from the PSG, sensitivity was 58.36% and specificity was 46.79%. While N3 did occur in the head low posture, the majority of HL posture epochs were scored as N2 (Fig 2a).

Lying posture was also not useful to identify stages of light sleep (N1 & N2) in the UK group. Of epochs when the cow was lying with the head up and still (UP) PPV was only 18.66% and NPV was 68.14%. Of epochs scored as either N1 or N2, posture correctly identified sleep stage in only 14.75% of epochs.

We then investigated the ability of lying with head still and low (HL) to identify all epochs of NREM sleep combined (N1, N2 and N3). This improved PPV to 34.8%, and NPV was 65.92%. Of known sleep epochs, HL posture correctly identified 54.02% of NREM sleep stages and of epochs not in NREM sleep, specificity was 46.78%.

NZ Pasture managed cow results

Table 4 displays the positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity for each of the postural predictions for sleep stages in the NZ pasture-managed cohort. Of epochs in the tucked posture (T), the positive predictive value to identify REM sleep was 69.04%. Of epochs not in the T posture, NPV was high at 99.86%. Of all epochs scored from the PSG as being in REM sleep, sensitivity was 97.81%. Specificity was 97.27%. As shown in Figure 3, in the pasture cohort in NZ, 98% of REM epochs occurred when the cow was in a tucked posture. The cows were awake in 16% of tucked posture epochs, and in other sleep stages the rest of the time. No REM sleep occurred in the flat-out posture (FO) or head resting front (HF) postures in the NZ cohort.

Table 4. Positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of the New Zealand outdoor managed cows describing the use of specific lying posture's ability to identify sleep stages.

Posture- Sleep Stage Identifier	*PPV*	*NPV*	*Sensitivity*	*Specificity*
Tucked Posture= REM	69.04%	99.86%	97.81%	97.27%
Resting Posture= REM	63.08%	99.86%	97.81%	96.44%
Head Low= N3	4.41%	94.40%	29.70%	64.76%
Head UP= N1/2	21.96%	82.99%	59.73%	48.08%
Head Low= NREM	23.49%	74.43%	33.06%	64.42%

Adding all head resting postures did not improve identification of REM sleep in the NZ cohort and decreased PPV (63.08%), and specificity (96.44%) slightly from the tucked posture scores.

Lying in the HL posture correctly identified epochs of N3 sleep in only 4.41% of epochs of HL posture (PPV). The NPV of epochs not in HL posture to identify epochs not in N3 sleep was 94.40%. Of epochs in N3 sleep, sensitivity of HL posture to correctly identify N3 was 29.7%. Of epochs scored in other sleep stages, the probability of other postures ruling out N3 (specificity) was 64.76%. As seen in Figure 3a, 31% of head low posture epochs were awake, and 33% were scored in N2.

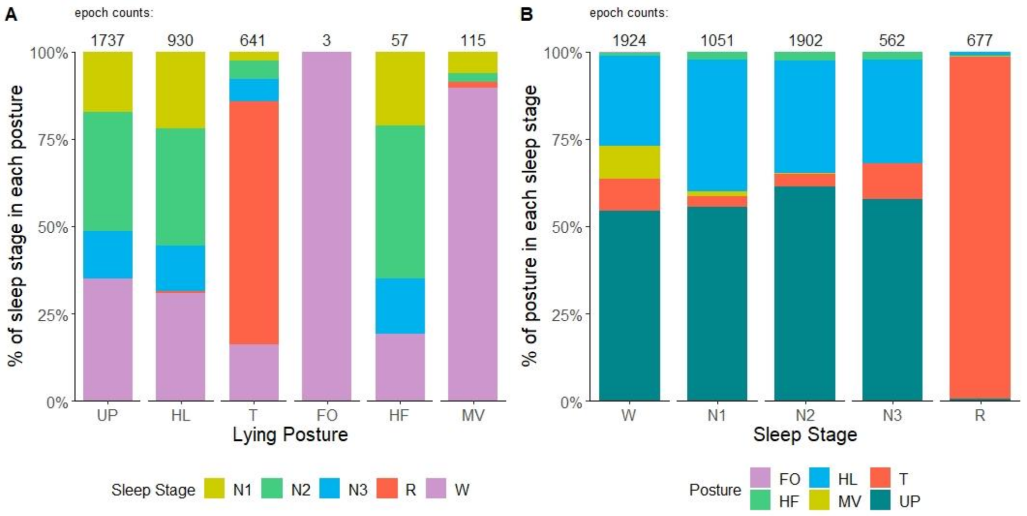

Figure 3 Proportions of sleep stage epochs that occurred in each lying posture in the New Zealand outdoor managed group (n= 6 cows) with total epoch counts of each posture above each bar (a), and proportions of lying posture epochs that occurred in each sleep stage in the outdoor managed group with total epoch counts of each sleep stage above each bar (b). Figure produced in R version 4.0.5 using ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/index.html)

Lying with the head up and still was also only able to positively identify 21.96% of N1/2 epochs. Using head low posture to identify all NREM sleep stages (N1, N2 & N3) did not improve PPV, and it was able to identify only 23.49% of NREM epochs correctly.

Individual differences

Within the UK group a degree of individual differences in lying postures during REM sleep occurred. Of the 4 cows included in the analysis, PPV ranged from 19.05% to 62.72%, NPV was consistently very high, but sensitivity ranged from 39.47%-96.13%. All cows in this group exhibited REM sleep during a flat-out lying posture, and one cow spent the majority of their REM sleep in this posture. REM sleep almost exclusively occurred in the tucked posture in the NZ cohort.

Discussion

In the UK indoor housed group, the tucked lying posture was not a strong identifier for REM sleep. Arguably, in this case, PPV and NPV are some of the most important ways of evaluating the success of using postures as a test for sleep stage, as they indicate the actual proportion of instances of the sleep stage that occur during observations of the lying posture as well as the inverse of this (Trevethan, 2017). Only 44% of instances of all epochs of tucked posture observed in the UK group were scored as REM sleep. Therefore, if this posture was used as a tool to identify REM sleep in cows in this type of environment, it would greatly overestimate the actual REM sleep time. Posture was a better indicator of REM sleep in the NZ pasture-managed group where REM sleep almost always occurred in the tucked posture, and as such the test had a high sensitivity. The PPV was still only 69%, indicating that using tucked posture would also overestimate actual REM sleep in this environment. All other stages were also found to occur in the tucked posture, particularly awake (W) and N1, although these other stages could have occurred before or after actual bouts of REM sleep. NPV and specificity of lying with the head tucked and lying with the head resting was high in both indoor and outdoor cows, showing that if a cow isn’t lying in one of these postures, they likely are not in REM sleep. However, this is not particularly useful when the goal is to identify instances when they are. These results are similar to those found by (Ternman et al., 2014), with indoor housed adult cows, who found low to moderate agreement of PSG with behavioural observations where for 30 second intervals, lying with the head resting had a sensitivity of 70-80% and specificity from 40-50%. PPV and NPV were not presented in that study.

Lying postures were also not useful to identify instances of NREM sleep in both groups. We tested the hypothesis that lying with the head still and low could be used to identify deep NREM (N3) sleep stages as with deepening sleep stages, there is a reduction of neck muscle tone on the EMG trace (Hänninen et al., 2008) and that lying with the head lifted still and up would be an indicator of lighter NREM sleep (N1 & N2). In both groups, however, the head low posture positively predicted only 6% (UK) and 4% (NZ) of N3 sleep epochs correctly. Lying with the head still and held up was also not a good indicator of N1 and N2 sleep stages combined. Interestingly however, the majority of NREM sleep occurred in the head up posture in the NZ group, while the majority of NREM sleep occurred in the head low posture in the UK group. We found that stages of NREM sleep occurred across almost all lying postures, but the awake stage also occurred in almost every lying posture as well. We did not score eye opening or closure from the video, as despite the 4 angles of the test pen captured on video, we could not reliably discern if the eyes were open or closed during night-time infra-red video recordings. This may have been a way to identify instances when the cow was lying in a posture but was awake as was used by Klefot et al. (2016) in a study attempting to compare behavioural sleep indicators to accelerometer data. However, it is also possible for the cow to be awake whilst keeping the eyes closed and indeed asleep with eyes partially open.

Despite PSG being the gold standard for sleep scoring and being the most reliable way of accurately identifying sleep in dairy cows, the difficulty of using PSG with these animals reinforces the need for an accurate alternative method that is more easily applied in a farm setting.

In this study, we considered each epoch to be independent of all others. While this makes sense for analysis and comparison between the PSG and lying postures, it is not indicative of what is occurring in real life. The epochs are in a time series, and sleep stages and postures can be grouped into bouts. Comparing behaviour with sleep stage over longer periods of time or considering recent preceding behaviour may increase accuracy. For example, a cow who was standing and immediately adopted a head resting posture upon lying down may be less likely to be in REM sleep than a cow who has been lying for a long time.

Interestingly, lateral lying was very rarely observed in the pasture cow cohort in NZ. When observed, it was always brief and tended to occur as a stretch or transition between other postures. This is different to results published by Krohn and Munksgaard (1993) who found cows on pasture spent more time lying on the side (flat-out/lateral lying) than when indoors in deep bedding. However, the cows in that study were usually managed indoors and may have taken advantage of the availability of space to engage in postures that would be restricted in their typical cubicle housing environment. This may then be similar to the UK cows who, prior to being included in our study, were managed with the milking herd which had access only to cubicles equipped with mattresses and light sawdust bedding. Therefore, these cows could have been showing long term rebounds from poor housing conditions restricting their ability to sleep in more expansive positions. Access to a larger unrestrictive lying surface could have influenced the postures achieved during sleep.

The NZ cows almost only adopted a tucked (T) posture during REM sleep, however, the NZ study occurred during the winter. The overall average temperature during daytime recordings was 10.2°C, with an average low of 5.3°C to an average high of 14.4°C (range 2 -18°C). During the night-time recordings, the overall average temperature was 8.4°C with an average low of 6.2°C to an average high of 11.4°C (range 2-14°C). A previous study found cows managed outdoors in winter in NZ were less likely to lie down in a tucked posture than when inside and were more likely to lie in a position that exposed less surface area to the elements when lying outdoors in cold weather (Tucker et al., 2007). Moreover, a tucked posture has been shown to conserve heat and protect the cow from wind (Schütz et al., 2010). The UK study was conducted from early spring to summer in the Dumfries and Galloway region in Scotland. Average daytime temperature during recordings was 15.2°C, with an average low of 11.3°C and an average high of 17.3°C (range 8-22°C). Over night-time recordings, the average temperature was 11.2°C with an average low of 8.4°C and an average high of 15.4°C (range 5-22°C). These temperatures indicate the outdoor climate, as temperature was not measured indoors, and it is possible that the cows would have experienced warmer temperatures due to insulating properties of the barn and deep bedded straw surface. Some of the differences in lying postures during REM sleep between groups could have been due to temperatures and cows seeking to minimize or maximize heat loss depending on temperature and lying surface.

In addition to temperature, NZ cows were also subject to the effects of wet weather which affected the lying surfaces. Cows prefer clean and dry bedding to wet and dirty lying surfaces (Schütz et al., 2019) and while we moved the test and group pens within a larger paddock to avoid wet and dirty conditions, wet lying surfaces occasionally occurred, which could have affected the cow's comfort and inclination to adopt more expansive lying postures.

Sleep is an instinctive behaviour, and with the accompanied behavioural inactivity and loss of vigilance, most animals are likely to choose sleeping locations that are secure, and thermodynamically favourable (Lima et al., 2005). Wild cattle are a prey species, and it is possible that despite the lack of predators in New Zealand, their domesticated cattle descendants have retained the instincts to locate secure and favourable sleeping locations. Pigeons, who prefer to sleep on higher perches, exhibited reduced REM sleep when forced to sleep on the ground, presumably due to perceived predator risk (Tisdale et al., 2018). It is possible that the cows managed on pasture adopted more upright and tucked positions to maintain a level of vigilance and ability to react quickly to threats. In contrast, cows in a sheltered barn may feel more secure or protected and therefore more likely to adopt flat out positions or to relax more fully and adopt positions that might take more time to react and rise from.

Stage of pregnancy and position of the calf could have also been a factor influencing lying posture during sleep. Advanced pregnancy has been found to have an effect on cow lying laterality (Forsberg et al., 2008). The NZ pasture cows were in their mid-late pregnancy during the recording period whereas the Scotland cows were not pregnant. It is possible that the presence of the growing foetus affected the cow's choice of lying posture or lying comfort in different postures.

Conclusion

REM sleep in dairy cows almost always occurs with the head resting or tucked, however, observation of the posture is not an accurate way of identifying REM sleep. Lying with the head lifted, immobile and held high or low was also not a good predictor for the occurrence of light and deep NREM sleep. Despite the limited sample sizes in these studies, there was variation between study environments and individual cows, so generalizations of sleep based on a specific posture may be inaccurate for some cows and when comparing between groups. PSG is a time consuming and impractical method for sleep identification in dairy cows, however using lying postures and head position is not likely to be a useful alternative. Investigations into more reliable and easily applied physiological alternatives for identifying sleep stages in dairy cows is necessary for the study of sleep in cows in the future.

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Declaration of Competing Interest:

The authors report no declarations of interest.

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CHAPTER FOUR

Heart Rate changes with sleep stage and lying posture in dairy cows

Preface

This chapter consists of a journal article that, since the submission of this thesis, has been published in the journal *Animals*. The chapter is structured in the *Animals* style however the formatting has been modified to fit with the style of this thesis and may differ slightly from the published version.

Hunter, Laura B, Haskell, M.J., Langford, F.M., Connor, C.O., Webster, J.R., Stafford, K.J., 2021. Heart Rate and Heart Rate Variability Change with Sleep Stage in Dairy Cows. Animals 11. https://doi.org/https://doi.org/10.3390/ani11072095 Academic

As discussed in the previous chapter, lying posture was not a useful proxy to identify detailed stages of sleep in dairy cows. Therefore, further investigation into other minimally invasive proxies for PSG to identify sleep stages in dairy cows are needed. As identified in chapter 1, HR and HRV are used in human sleep stage detection and can be a minimally invasive method to identify sleep. In cows as with humans, Polar heart rate monitors are often regarded as being the most accurate monitoring tools compared with ECG, particularly for measuring inter-beat intervals (IBI) or R-R intervals (Figure A) which are needed to calculate HRV.

The aim of this chapter was to determine if HR and HRV measures changed with sleep stage to determine their suitability for sleep stage assessment in dairy cows. Furthermore, we aimed to investigate differences in the HR an HRV of the housed cows in Scotland and pasture based cows in New Zealand and determine if lying posture affected also affected the heart rate.

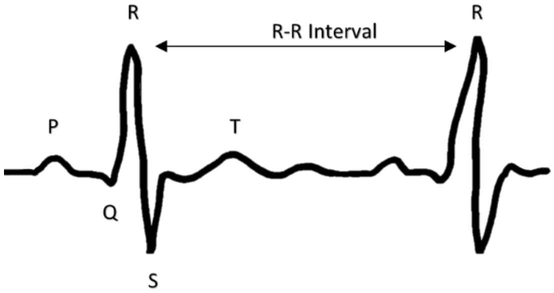

Figure A. Diagram of two heart beats indicating the individual elements (PQRST) of each heartbeat as well as inter-beat interval or R-R interval.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Laura Britney Hunter
Name/title of Primary Supervisor:	Professor Kevin Stafford

In which chapter is the manuscript /published work: 4

Please select one of the following three options:

○ The manuscript/published work is published or in press

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Animals

- The percentage of the manuscript/published work that was contributed by the candidate: 85.00

- Describe the contribution that the candidate has made to the manuscript/published work:

Laura had a primary role in the project conception, data collection, statistical analysis, interpretation of the results and writing of the paper with supervision from supervisors.

○ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Laura Hunter
Date:	21/6/21
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Date:	21/6/21

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Heart rate and heart rate variability change with sleep stage in dairy cows

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Simple Summary:

The amount of sleep acquired and changes to patterns of sleep could be a useful tool to assess cow welfare, particularly in response to changes or stressors in their environment. However, the current most accurate method to assess sleep, Polysomnography (PSG), is difficult and time consuming. In humans, the heart rate (HR) and variability in time between heart beats (HRV) can be used to identify sleep stages, and this could be a useful alternative to investigate sleep in cows. We compared measures of HR and HRV with PSG in two groups of dairy cows in different environments and investigated the effects of lying posture on these measures. We found that HR decreased with deepening sleep stages in both groups of cows, that rapid eye movement sleep (REM) was associated with higher HRV and that HR and HRV also changed with different lying postures. The patterns of the differences between sleep stages were similar between two

groups of cows. Our results suggest that HR and HRV change with sleep stages in cows and that these measures could be a useful, and more easily applied, method of assessing sleep stages in dairy cows.

Abstract:

Changes to the amount and patterns of sleep stages could be a useful tool to assess the effects of stress or changes to the environment in animal welfare research. However, the gold standard method, Polysomnography PSG, is difficult to use with large animals such as dairy cows. Heart rate (HR) and heart rate variability (HRV) can be used to predict sleep stages in humans and could be useful as an easier method to identify sleep stages in cows. We compared the mean HR and HRV and lying posture of dairy cows at pasture and when housed, with sleep stages identified through PSG. HR and HRV were higher when cows were moving their heads or when lying flat on their side. Overall, the mean HR decreased with depth of sleep, there was more variability in time between successive heart beats during REM sleep, and more variability in time between heart beats when cows were awake and in REM sleep. These shifts in the HR measures between sleep stages followed similar patterns despite differences in mean HR between the groups. Our results show that HR and HRV measures could be a promising alternative method to PSG for assessing sleep in dairy cows.

Keywords: Dairy cows, heart rate, sleep, heart rate variability, polysomnography

Introduction

Two main stages of sleep exhibited by animals are known as rapid eye movement sleep (REM) and non-REM sleep. Non-REM sleep has been associated with restorative functions in the body and brain, for example, the clearance of potentially harmful toxins produced by normal cellular function (Xie et al., 2013). REM sleep has been associated with memory, learning and dreaming

(Le Bon, 2020). Changes to the amount and patterns of sleep stages could be used to assess animal welfare, as these aspects of sleep are known to be affected by factors such as environmental conditions, stressful occurrences during the day, pain or illness (Langford and Cockram, 2010). However, in dairy cows, without using neuro-electrophysiological methods, it is difficult to accurately identify sleep from wakefulness, let alone different sleep stages.

As sleep is a homeostatic function originating in the brain, the most accurate way to study it is through polysomnography (PSG), the study of multiple electrophysiological signals, namely brain activity, eye movements and muscle activity (Carley and Farabi, 2016). PSG can be successfully used to study sleep in cattle, however it is costly, the equipment is fragile, and interpretation of the signals is time consuming (Ternman et al., 2012). Being able to identify sleep in dairy cows with other more easily applied or less invasive devices would be beneficial not only for the cow's comfort and welfare, but also for ease of application by researchers, thus facilitating the study of the sleep of cows and opening several new avenues for investigation of the effects of sleep loss or importance of sleep for cows.

During sleep and the different sleep stages, changes occur in the regulation of the mammalian autonomic nervous system (ANS) and its subdivisions - the parasympathetic (PNS) and sympathetic nervous systems (SNS), affecting many functions such as heart rate, respiration rate and body temperature (Zoccoli and Amici, 2020). Specifically, during REM sleep, there is variability in the ANS activity, leading to more variability in the associated physiological functions whereas in non-REM sleep stages there is more activity of the PNS while SNS activity is reduced (Zoccoli and Amici, 2020). Because the ANS affects the heart, measures of heart rate (HR) and heart rate variability (HRV: the measurement of the variability in the time between successive heart beats), can be used as a way to identify activation of the PNS and SNS (Bertsch et al., 2012). In humans, the changes in HR and HRV have been used to accurately identify and differentiate between sleep stages (Mitsukura et al., 2020; Xiao et al., 2013). HRV can be quantified with different methods. Time domain indices of HRV identify differences in the time between successive heart beats or inter-beat-interval (IBI) while frequency domain indices classify the signal into frequency bands (Shaffer and Ginsberg, 2017).

The study of HRV in cow welfare to date has focussed mainly on the application of HR and HRV to identify and assess stress. HR and HRV were found to be affected by severe lameness which may cause chronic stress in cattle (Kovács et al., 2015). Calves being disbudded without local anaesthetic showed an increase in frequency domain metrics of HRV (Stewart et al., 2008). HRV has also been used to identify positive interactions in dairy cows as well, social licking between cows was found to reduce heart rate in receivers (Laister et al., 2011). Body posture has been found to affect HR and HRV measures, the heart rate was lower and variability in time between heart beats was higher in cows lying down compared to when standing (Frondelius et al., 2015a), but investigations of HR and HRV during sleep in cows have not been done. In previous work, we have found that sleep occurs when cows are lying down but that specific lying postures could not be used reliably to identify sleep stages compared to PSG in dairy cows and that housing conditions have been shown to affect the relationship between lying postures and sleep (Hunter et al., 2021b).

It is possible that HR and HRV could be used to identify sleep stages in cows. The equipment required to assess HR and HRV is a less invasive and more easily applied than used for PSG. Therefore, the objective of this study was to determine if HR and HRV differ between sleep stages in dairy cows, and, to determine if this is repeatable between cows in different areas, housing conditions and lying postures.

Materials and Methods

Ethical Statement

The study was designed in accordance with the relevant guidelines and legislation in both Scotland and New Zealand (NZ) where the studies took place. Ethical approval was obtained from the UK Home Office (Project Licence P204B097E), SRUC Animal Ethics Committee (Ref. ED AE 03-2018) and Ruakura Animal Ethics committee (AE 14708) prior to the start of animal manipulations.

Cows and Housing

Twelve cows were recruited for this study from two locations. Six non-lactating and non-pregnant Holstein cows (age 3.86±0.68) from the Acrehead unit of SRUC's Dairy Research Centre (Dumfries, Scotland) and six, three-year-old, non-lactating, pregnant Kiwi-cross (Friesian x Jersey) cows from the DairyNZ Lye Farm (Newstead, NZ) were used. Non-lactating cows were selected to avoid disruptions to the cow's sleep patterns due to fetching for milking and the risk of damage to recording devices in the milking parlour. The Scottish cows, destined to 'retire' from the herd due to reduced fertility, were healthy during the trial.

The cows were managed in a large group pen. and moved into a smaller adjacent 'test' pen individually for recording sessions (Figure 1). The Scottish cows were held on deep-bedded straw in a barn. The group pen measured 20m x 5.2m and test pen 5.2m x 5.05m. The cows were fed silage and always had access to water. The NZ cows were managed outdoors in a large paddock. They were able to graze and were provided with silage *ad libitum* and always had access to water. The group pen measured 44m x 29m and was created with live electric fencing. The 10m x 10m test pen was created with non-live electric fencing tape, to prevent potential interference with the electrophysiological recordings. The fencing set-up for both group and test pens could be moved around the paddock when ground conditions became wet or muddy. In both locations, a 2m buffer zone was created between the test pen and group pen, to inhibit contact and reduce damage to recording devices from social interactions.

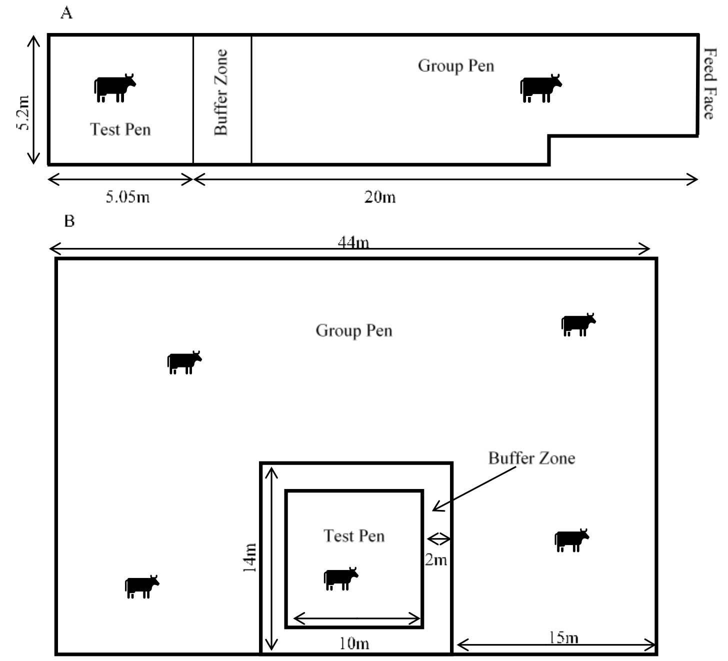


Figure 1 Diagrams of group and test pen design in the Scottish indoor housed study (A) and in the NZ outdoor pasture study (B). During recordings, the test cow was moved into the test pen. When not recording, the cow was moved back into to the group pen.

Data Collection

The cows were fitted with the recording devices and moved into the test pens individually for a maximum of 7 days. The devices and recording gear were downloaded, re-charged and re-set twice daily. Devices were removed if the cow showed signs of skin or behavioural irritation or in the case of forecasted heavy rain (NZ outdoor group).

Heart Rate Recording

HR and HRV were measured using a polar RS800 CX watch and Polar equine monitoring belt (Polar Electro Oy, Kempele, Finland). Patches of hair at the electrode locations were clipped and the electrodes were generously coated with ultrasound gel (Aquasonic 100 gel, Parker Laboratories, NJ, USA) to improve the contact with the skin and signal transmission. The belt and watch were checked frequently and adjusted as needed throughout the recording. An elastic surcingle was attached over the belt to keep it tight to the skin. The clasps of the Polar belt and surcingle were padded with felt and wrapped in cohesive bandage to reduce chances of irritation to the cows and also reduced the chance of the surcingle loosening throughout the recording. The watch was synchronized to the recording computer's time and was programmed to record heart rate and R-R intervals which are used for HRV calculations. R-R intervals are the time (in milliseconds) from the R peak of one heartbeat to the R peak of the next heartbeat.

The data were downloaded and analysed using Polar Pro Trainer (Version 5.35.160) and artefacts in the R-R data were filtered and corrected using the moderate filter power. Only traces containing less than 1% of identified errors were used in the analysis. Filtered data were exported and HR and HRV statistics were calculated in 30 second intervals (epochs) corresponding to the scored PSG data. Only time domain features of the HRV were calculated since frequency domain features of the HRV may not be an accurate representation of the data in such small time periods (Bourdillon et al., 2017). Time domain features included mean HR (in beats per minute - BPM), root mean square of successive differences of the R-R signal (RMSSD), and standard deviation of the R-R signal (SDRR) in 30 second epochs.

PSG Recording and Sleep Scoring

PSG recording protocols

PSG were recorded as described in Hunter et al., (2021a). Pre-gelled adhesive snap ECG electrodes (Natus Neurology, Kanata, Canada) were used to record four EEG, a reference (REF), patient grounding (PGND), two EOG and two EMG channels from the cows. Lead wires were snapped on, bundled down the neck and plugged into the Embletta MPR PG +St proxy PSG recording device (Embla Systems, Kanata, Canada). The device was placed in a padded plastic box within a pouch sewn to the elastic surcingle covering the HR monitor belt. The device was programmed, data were downloaded, and traces processed and scored using RemLogic 3.4.3 software (Embla Systems, Kanata, Canada). Good quality recordings, which had a minimum of one complete EEG, EOG and EMG trace each with good impedance (1-14kHz), minimal artefacts and with good quality corresponding HR traces were used in the analysis. Recordings lasted a maximum of 10 hours due to device memory limitations.

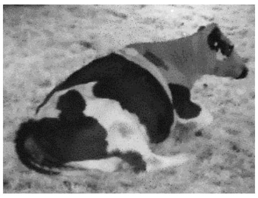
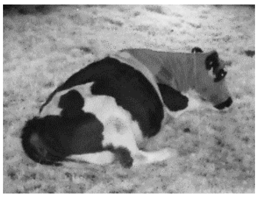
Sleep Scoring

The good quality traces were scored according to criteria developed from a combination of previous cow sleep EEG studies (Hänninen et al., 2008; Ruckebusch et al., 1970; Ternman et al., 2012) and well as human sleep scoring criteria (Iber and American Academy of Sleep Medicine, 2007). Five stages of sleep and wakefulness were scored: Awake (W), REM (R) and Non-REM (which was subdivided into 3 stages, light N1 and N2, and deep N3). Rumination was also scored from the PSG as substantial artefacts due to jaw muscle movements when chewing obscured the actual signals of the traces and it was impossible to tell what stage the cow was in during that time. Intra-observer accuracy was calculated using “irr” package (Gamer et al., 2019) in R (Version 4.0.5) using Cohen’s Kappa with $\kappa = 0.83$ and overall agreement of 89.4% indicating good agreement (McHugh, 2012).

Lying Postures

Lying postures were identified from video recordings made from four surveillance cameras equipped with automatic infra-red night vision capability (Geovision monitoring system, Viewlog, GeoVision Inc., Taiwan (Scottish Cows)) and Vivotek ND9541P H.265 NVR (Vivotek Inc., Taiwan) (NZ Cows)). Lying postures; with head held up above the point of the shoulder (UP), head held below the shoulder (HL), head resting on the ground to the front (HF), neck turned with head resting on flank or “tucked” (T), lateral lying or “flat out” (FO) and moving (MV) as well as not scored (NS) (Table 1) were scored instantaneously every 30 seconds corresponding to the start of the PSG and HR epochs. Intra-observer reliability was conducted in R (version 4.0.5) (R Core Team, 2021) using the Cohen's kappa in the `irr` package (Gamer et al., 2019) and the kappa statistic was κ = 0.95 demonstrating a high level of agreement (McHugh, 2012).

Table 1 Behavioural ethogram for scoring lying postures in dairy cows, including head positions and photographs from surveillance videos.

	**Head Up (UP)** Lying sternally recumbent with head held up
	**Head Low (HL)** Lying sternally recumbent with head held low
	**Head Resting Front (HF)** Lying sternally recumbent with the head and or neck resting on the ground
	**Tucked (T)** Lying sternally recumbent with head turned and resting on the flank
	**Flat-out (FO)** Lying laterally with legs extended and head and neck resting on the ground

Data Analysis

Scored sleep stages, lying postures and heart rate data were aligned by time stamps. In cows, sleep occurs when lying down (Ruckebusch et al., 1970), therefore only epochs identified as lying were included in the analysis. Epochs with posture 'not scored' (NS) due to observer inability to accurately observe behaviour or other extraneous circumstances were also removed from the dataset. As the stages of sleep or wakefulness could not be determined while ruminating, these epochs were also removed.

We fitted a mixed effects model to determine if the cow's HR changed by sleep stage using the 'lme4' (Bates et al., 2015) and 'lmertest' (Kuznetsova et al., 2017) packages in R (version 4.0.5) (R Core Team, 2021). The fixed effects were study (country), sleep stage and their interaction. We included recording day nested within individual cow ID as random factors. We then used the same model with each of the remaining variables; RMSSD and SDRR as the response variables. Using the 'predictmeans' (Luo et al., 2021) package we calculated the predicted means, standard error of the means (SEM) and least significant differences (LSD).

We then re-ran the same models, now including the cow's lying posture as a fixed effect with interaction with study and calculated predicted means of cow's HR and HRV measures by lying posture and study.

Results

Overall, with rumination, standing, and unscored lying behaviour removed, 1968 epochs totalling 16.4 hours of good quality data were obtained from 10 cows in 29 recordings days. Data from one Scottish and one NZ cow were removed as they each had only one limited good quality recording that did not contain any lying periods. The data set was skewed towards more time in the awake (W) state, as 629 epochs were scored as W, 315 epochs in N1, 593 epochs in N2, 197 epochs in N3 and finally 234 epochs in REM (Table 2).

Lying Posture

The HR and HRV parameters changed with specific body posture while lying. In the Scottish cows, moving and lying flat out postures resulted in significantly higher mean heart rate (MV=

56.43 ±3.17 bpm, FO= 55.53 ±3.15 bpm), than all other postures (lying with the head up, or low, or resting on the ground or with the head tucked) (Figure 2). Flat out lying was rare in the NZ data, with only one epoch over all observations. Moving was also associated with a higher mean HR in the NZ group (84.22 ±3.41 bpm). In the NZ cows, tucked posture was also associated with significantly higher RMSSD values than the head low, head up and moving postures indicating more variability in the time between successive heart beats. Similar results were found with RMSSD in the Scottish group, who had higher RMSSD in T compared to HL (p=0.007), UP (p=0.0017) and lower compared to FO (p=0.0035). We also found a significant effect of sleep stage and its interaction with study location on the HR and HRV parameters**.** Table 2 shows the means for the different sleep stages.

Table 2 Count of the total number of epochs of data in each posture by sleep stage (tucked (T), head resting front (HF), head low (HL), head up (UP), moving (MV) and flat out (FO)) and study country.

	NZ							Scot						
	T	HF	HL	UP	MV	FO	Total	T	HF	HL	UP	MV	FO	Total
W	24	2	67	116	14	1	224	42	4	133	157	49	20	405
N1	4	2	34	58	3	0	101	20	1	103	52	13	25	214
N2	9	4	61	120	0	0	194	51	13	217	46	6	66	399
N3	8	2	21	47	0	0	78	17	1	72	15	3	11	119
R	90	0	0	1	0	0	91	114	1	4	3	1	20	143
Total by Posture	135	10	183	342	17	1	688	244	20	529	273	72	142	1280

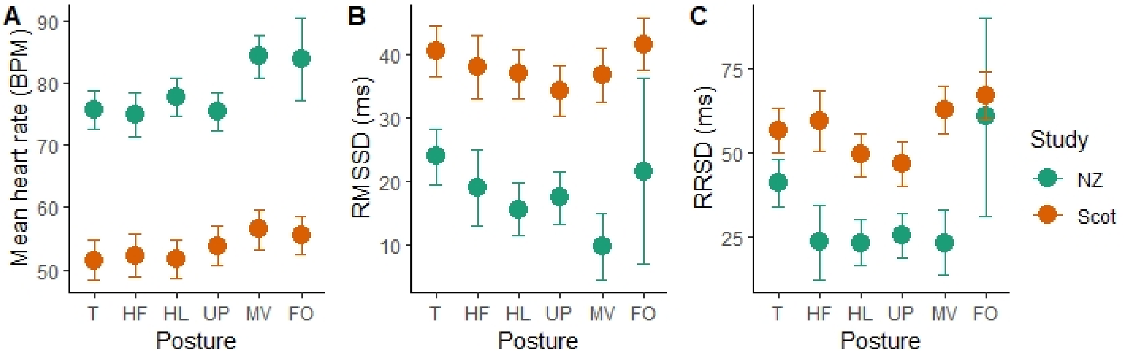


Figure 2. Plots of predicted means with error bars indicating standard error of the means for the mean heart rate in beats per minute (bpm) (A), the RMSSD (B) and SDRR (C) by lying postures (Tucked (T), head resting front (HF), head low (HL), head up (UP), moving (MV) and flat out (FO)) for the New Zealand group (NZ), Scottish group (Scot). Figure produced in R version 4.0.5 using ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/index.html).

Mean HR

After accounting for variation between cows and study days, we found a large effect of study group on the mean HR. The mean HR were around 20 BPM lower in the Scottish cows than the NZ cows. After accounting for this variation, significant differences between sleep stages were evident. In both the indoor-housed Scottish group and outdoor-managed NZ cows, mean heart rate was significantly slower in the REM sleep stage compared to awake (W) (NZ: p<0.001) (Scottish: p<0.001). In the Scottish group, N2 and N3 stages were not significantly different from one another (p=0.09), but all others (W, N1, R) were. In the NZ group, W and N1 were not different from each other (p= 0.46), and neither were N3 and REM (p= 0.89). Overall, heart rate declined successively from W to N1 and then to N2, while N3 and REM were significantly lower than the other sleep stages.

RMSSD- variability between successive heart beats

As heart rates were significantly different between the study groups, it is unsurprising that they also had a significant effect of study on the RMSSD (Figure 3). As the mean heart rate was lower in the Scottish group, their RMSSD was 15-30ms higher than the NZ group indicating longer inter-beat intervals (Table 3). Accounting for the random effects, we found significantly higher RMSSD values during REM sleep epochs, indicating more variability in the time between successive heart beats in this stage. In the NZ group, the RMSSD during REM sleep was significantly higher than W (p=0.0061), N1 (p= 0.01) and N2 (p=0.0056) but not significantly different from N3 (p= 0.18). In the Scottish group, RMSSD during REM sleep was highly significantly different from all other sleep stages which did not differ greatly from one another. However, N2 did differ significantly from W (p=0.002) and N1 (p = 0.028). Overall, the time between successive heart beats during REM sleep was significantly more variable than the other stages. N3 was more variable than W but not compared to the other NREM stages (N1 & N2).

SDRR- total variability of time between heart beats

There were differences between the groups, but this was not as wide as for the other variables. SDRR was higher for the awake and REM stages compared to the other stages, indicating that there was higher variability in the overall time between heart beats for these stages. In the NZ cows, SDRR during REM sleep was significantly higher than N3 (p=0.0288), but not the other stages. N3 was significantly lower than W (p= 0.0337), however N3 was not significantly different from the other stages. In the Scottish group, SDRR was not significantly different between W and REM (p=0.4422), but these stages were significantly higher than N1, N2 and N3 that were not significantly different from one another.

Table 3 Table of predicted means ± standard error of the HR mean, RMSSD and SDRR for each of the stages, awake (W), Non-REM: N1-N3 and REM sleep overall data and by study group in NZ and Scotland (SC).

Sleep Stage	W	N1	N2	N3	REM
HR Mean					
Predicted Mean NZ	78.03^{A}	78.57^{A}	76.72^{B}	73.90^{C}	73.78^{C}
	± 3.05	± 3.09	± 3.06	± 3.10	± 3.09
Predicted Mean SC	56.77^{D}	53.56^{E}	52.20^{F}	51.14^{F}	49.48^{G}
	± 3.04	± 3.05	± 3.04	± 3.08	± 3.07
RMSSD					
Predicted Mean NZ	17.15^{A}	16.80^{A}	17.14^{A}	19.22^{AB}	22.14^{BC}
	± 3.97	± 4.12	± 3.99	± 4.18	± 4.14
Predicted Mean SC	32.92^{CD}	33.41^{DE}	36.03^{F}	35.96^{EF}	50.96^{G}
	± 3.82	± 3.88	± 3.83	± 3.98	± 3.94
SDRR					
Predicted Mean NZ	31.74^{AB}	30.95^{ABC}	26.30^{AC}	23.32^{C}	33.08^{ABD}
	± 6.22	± 6.60	± 6.27	± 6.75	± 6.63
Predicted Mean SC	62.93^{E}	49.61^{D}	46.69^{BD}	44.83^{BD}	60.71^{E}
	± 6.03	± 6.19	± 6.04	± 6.42	± 6.34

$^{A\text{-}G}$ Means without a common superscripted letter are significantly different at $p<0.05$

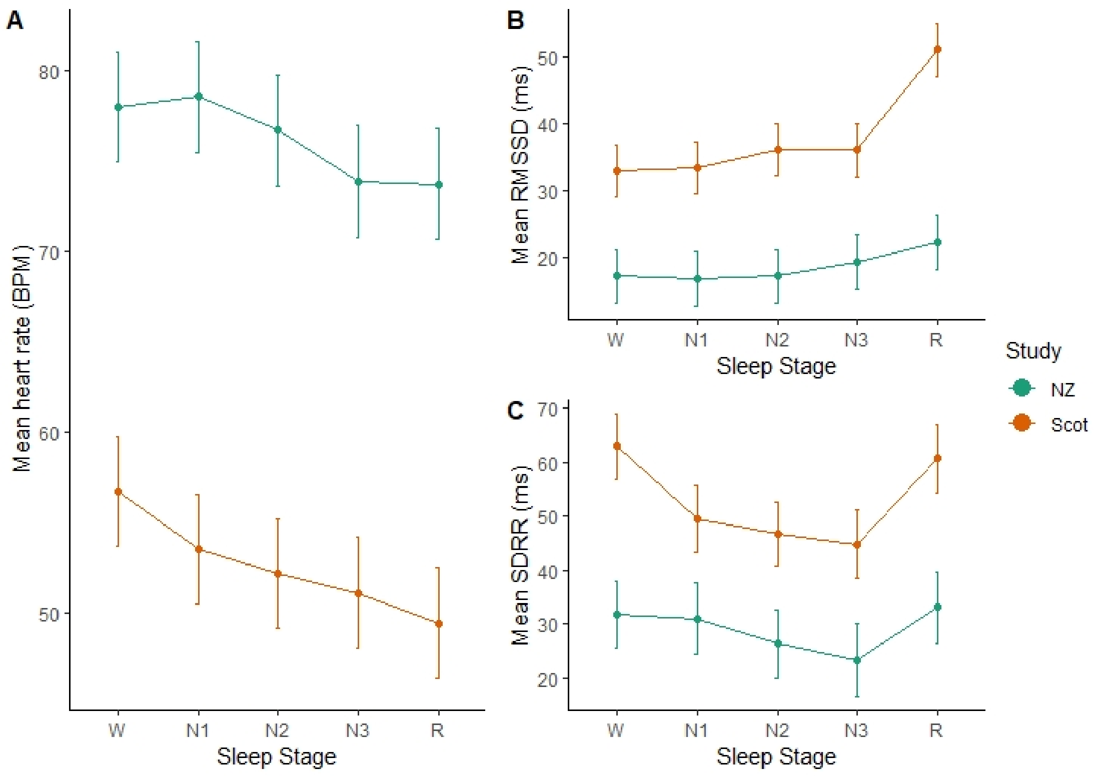


Figure 3 Plots of predicted means with error bars indicating standard error of the mean, in each sleep stage for the NZ and Scotland groups for the mean HR (A), RMSSD (B) and SDRR (C)). Figure produced in R version 4.0.5 using ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/index.html).

Discussion

Our results show that cardiac outputs could be useful in assessing sleep stages in dairy cows. However, we found major differences in mean HR between the two groups of cows, these may be due to the different cow characteristics. Understandably, despite replication in the data collection methods, there were marked differences in the housing, breed, size, physiological stage, and diet of the cows in each study location. The NZ cows were all in late pregnancy, whereas the Scottish cows were non-pregnant and non-lactating. Late pregnant heifers and cows have been found to have higher mean HR than earlier on in pregnancy (Trenk et al., 2015). While there was not a particularly large differences in cow age, there was a difference between cow size and breed. The Scottish cows were very large Holstein cows, and the NZ cows were much smaller being Jersey-Holstein crosses (Kiwi-cross). Other studies have found significant differences in HR and HRV measures between different breeds (Brown Swiss and Simmental) when standing, lying and milking (Hagen et al., 2005). Body size is also known to affect HR and HRV, and a decrease in HR was found with increasing weight in horses and ponies (Schwarzwald et al., 2012).

Importantly, despite these group differences, we found that HR and HRV changes with sleep stages in both groups and clearly, Figure 2 shows that the differences are in the same direction. These results indicate that the patterns of the changes in HR and HRV measures between the sleep stages are stable and as such these measures could be used with all cows, although further research is needed to assess if these patterns are also observed in lactating cows and cows in other stages of pregnancy.

Surprisingly, we found that mean HR during REM sleep was lower than when awake and in the lighter NREM sleep stages (N1 and N2). This is different to results in humans, where HR has been found to decrease with the progression of NREM sleep stages, and speeds up again in REM sleep (Cajochen et al., 1994). However similar results with an overall lower heart rate during REM sleep were also found in dogs (Varga et al., 2018). Despite the lower mean HR, HRV measures of RMSSD and SDRR were higher in REM sleep, indicating more variation between heart beats. This observation is similar to that shown for HRV patterns during human sleep, where HRV tends to be more variable when awake and in REM sleep than during N3 and other NREM sleep stages (Stein and Pu, 2012). Mean HR and RMSSD may be useful to distinguish between awake (W) and REM stages, however, they are not particularly useful to distinguish between NREM stages (N1, N2, N3). SSDRR was useful to identify N3 stages in both groups as it was significantly lower. These patterns of differences in sleep stage could be useful in future applications to predict sleep stage of dairy cows, particularly if prior to recording, a lying awake baseline could be specified. Then sleep stages could be identified or predicted from differences from that baseline.

A previous study has found that body position had a significant effect on HR and HRV measures in cows (Frondelius et al., 2015b), however they did not specify body posture while lying and were unable to identify awareness levels. We found that the specific posture that cows adopted during lying affected their HR and HRV, and in particular that epochs identified as being in the flat out (lateral lying) posture and epochs with the head moving resulted in higher HR and more variability in the HRV. As moving is a physically active behaviour, this activity may have had a carry-over effect on the heart rate for an extended period. So, an epoch in which the cow moved her head at the start may have higher HR across that epoch and into the next. Flat out posture was rarely observed in the NZ group, and only scored once, and even then, was only observed as a transition behaviour between other postures. In the Scottish group, flat out postures were far more commonly observed, and most often occurred while the cow was in N2 sleep as well as awake and in N1. It is unclear if the increased HR in this posture was due to the position of the body which could have facilitated a faster movement of blood, or because most epochs scored as flat out happened to occur in sleep stages that had higher heart rates. In the NZ group almost all REM sleep occurred in the tucked posture. The tucked posture was found to have significantly higher RMSSD, however since REM sleep was also found to have higher RMSSD it is likely that the effect of the posture on the HRV measure was more likely due to the sleep stage in this case.

The intra-observer reliability for sleep scoring was 89%, which according to inter-scorer reliability in human sleep studies is very good (Danker-Hopfe et al., 2009; Wendt et al., 2015) . However, there is still some possibility that the 11% uncertainty in sleep scoring was a contributing factor to the variability of the HR and HRV measures within sleep stages. Additionally, we analysed the HR data in 30 second epochs, specifically to correspond to the sleep scoring. The 30 second epoch is a standard practice in scoring sleep stages from PSG as it corresponds well to the structure of human sleep, containing fewer stage shifts than longer epochs which would be more likely to contain many stage shifts (Schulz, 2008). Despite shorter epoch length, some mid-epoch stage shifts could still have occurred. In these instances, although the PSG was scored one way, the HR measures could have reflected another stage, and this could also be another source of variability in the HR and HRV measures within sleep stage.

Similarly, the HR and HRV data may have also been influenced by the short epoch windows. Typically, human HRV measures are conducted in 5-minute increments, although ultra-short windows such as 30 second windows have been found to be acceptable for the assessment of HRV at rest in humans (Wu et al., 2020). Bouts of cow sleep stages can typically be quite short (Ruckebusch et al., 1970; Ternman et al., 2012) and thus multiple stage shifts would be captured in a longer epoch length of 5 minutes. This was a major reason for choosing to analyse the HR and HRV in ultra-short windows. However, some have questioned the accuracy of windows shorter than 2-minutes for the analysis of HRV in human athletes (Bourdillon et al., 2017). RMSSD measurements in even shorter 10 second windows were also found to correspond well to standard longer intervals in humans, but SDRR did not (Thong et al., 2003). Therefore, the short time window selection could have affected the accuracy of the cow HRV RMSSD and SDRR measurements. HR and HRV may be useful for the assessment of sleep stages in dairy cows, however, further investigation into the validity of ultra-short HRV measures in dairy cows and additional validation with PSG is needed.

Conclusion

We have shown that sleep stage sleep stage is associated with changes in HR and HRV in dairy cows. Mean HR was significantly lower in the indoor-housed, non-pregnant, and non-lactating cows compared to pregnant dry outdoor managed cows. We also found that mean HR decreased with sleep depth, SDRR was more variable in awake and REM and RMSSD was significantly higher in REM sleep than the other stages. These results indicate that HR and HRV could be a useful measure for the future identification of sleep stages in dairy cows using less invasive devices than PSG, making sleep research for animal welfare more accessible.

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Institutional Review Board Statement: The study was designed in accordance with the relevant guidelines and legislation in both Scotland and New Zealand where the studies took place. Ethical approval was obtained from the UK Home Office (Project Licence P204B097E), SRUC Animal Ethics Committee (Ref. ED AE 03-2018) and Ruakura Animal Ethics committee (AE 14708) prior to the start of animal manipulations.

Data Availability Statement:

*Data can be made available

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CHAPTER FIVE

Machine learning prediction of sleep stages in dairy cows from heart rate and muscle activity measures

Preface

The body of this chapter consists of a journal article published in *Nature's Scientific Reports*. The article has been re-formatted to follow the style of this thesis, however, retains the structure of the published paper.

Hunter, Laura B., Baten, A., Haskell, M.J., Langford, F.M., O'Connor, C., Webster, J.R., Stafford, K., 2021a. Machine learning prediction of sleep stages in dairy cows from heart rate and muscle activity measures. Sci. Rep. 11. https://doi.org/10.1038/s41598-021-90416-y

Machine learning involves programming a computer to learn from a data set and to make predictions based on statistical probability. Using 'supervised' machine learning methods, human input is required to 'teach' the computer how to recognize different categories. The data must first be labelled and then is split into a 'training set' and a 'test set'. The labelled training set is used to teach the model examples to learn to associate differences in the features and to correctly identify the label. The test set then uses unlabelled examples to evaluate how well the model has learned and how good its predictions are compared to the actual label.

Label*: The outcome variable or category of the data*

Feature: *The input variables*

Training Set: *Labelled data used to teach or train the model*

Test set: *Data used to evaluate the model (with labels removed)*

In the preceding chapter, I found that HR and HRV changed with sleep stage, however, using the HR and HRV alone to identify sleep stages in cows at this stage may be difficult due to large differences between the groups, and occasionally subtle differences between stages. As discussed in Chapter 3, behaviourally, the cow's neck position was not an ideal indicator of sleep stage in the cows, however, almost all REM sleep occurred when the cows had their heads resting. The neck EMG was a useful component for the visual identification of the sleep stages and the criteria developed in Chapter 2, thus, it follows that the raw EMG data also has the potential to be used to identify sleep stages in cows.

Therefore, the aim of this final experimental chapter was to investigate the potential to integrate several data sources using machine learning models to be able to predict sleep stage and to evaluate their success compared to PSG in dairy cows.

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Machine learning prediction of sleep stages in dairy cows from heart rate and muscle activity measures

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Abstract

Sleep is important for cow health and shows promise as a tool for assessing welfare, but methods to accurately distinguish between important sleep stages are difficult and impractical to use with cattle in typical farm environments. The objective of this study was to determine if data from more easily applied non-invasive devices assessing neck muscle activity and heart rate (HR) alone could be used to differentiate between sleep stages. We developed, trained, and compared two machine learning models using neural networks and random forest algorithms to predict sleep stages from 15 variables (features) of the muscle activity and HR data collected from 12 cows in two environments. Using k-fold cross validation we compared the success of the models to the gold standard, Polysomnography (PSG). Overall, both models learned from the data and were able to accurately predict sleep stages from HR and muscle activity alone with

classification accuracy in the range of similar human models. Further research is required to validate the models with a larger sample size, but the proposed methodology appears to give an accurate representation of sleep stages in cattle and could consequentially enable future sleep research into conditions affecting cow sleep and welfare.

Introduction

Animals are driven to sleep and it is vital that enough restful sleep is achieved to feel replenished (Siegel, 2005). Feelings of exhaustion, tiredness and sleeplessness can impact negatively on animal welfare (Mellor and Beausoleil, 2015). Health can also be significantly impacted by sleep loss (sleep deprivation or restriction), which can result in activation of the immune and inflammatory systems (Faraut et al., 2012) and also influence pain sensitivity and perception (Sardi et al., 2018) in both humans and animals.

We know very little about the importance of sleep and the effects of limited or poor-quality sleep for dairy cows. Broadly, it is likely that factors affecting lying behaviour will also influence sleep, as cows must lie down to achieve it (Ruckebusch, 1974). Sleep can be affected by stressful experiences during the day (Langford and Cockram, 2010). Therefore, changes to sleep patterns or total sleep time in cattle could be useful indicators for stress and other welfare concerns. The ability to identify sleep stages accurately could enable research on the effects of sleep loss for cows and could be useful to inform management practices such as determining rest intervals during long-haul transport or management of cattle during wet weather (i.e. on standoff pads).

Sleep consists of two main types: rapid eye movement (REM) and non-REM (NREM) sleep. The most accurate method of identifying sleep types is polysomnography (PSG)(Drinkenburg et al., 2016; Miano et al., 2010), which consists of a combination of physiological measurements; mainly electroencephalography (EEG), electromyography (EMG), and electro-oculography (EOG), which record electrical signals of the brain, as well as muscle and eye activity. Using specialized software, traces from these signals are analyzed and scored visually using characteristic patterns to determine sleep stages according to defined criteria. REM sleep is a deep sleep stage, where the brain is active, the muscle tone is low and there are often frequent eye movements. The majority of human total sleep time is spent in NREM sleep, which can be further divided by ‘depth’ into 3 stages from light - N1 and N2 sleep to deep N3 or slow wave sleep (SWS). SWS is characterized by high amplitude oscillating activity on the EEG accompanied by lower muscle tone and lack of eye movements. Many of the restorative functions of sleep are thought to occur in this stage (Xie et al., 2013). Dairy cows have been found to sleep for approximately 3-4 hours per day, but only around 30 minutes of this in REM sleep (Ruckebusch et al., 1970; Ternman et al., 2012). Therefore, most of the sleep time also consists of NREM sleep stages and it is likely that these stages serve important functions for cows as they do humans.

PSG has recently been used to record sleep in calves (Hänninen et al., 2008) and cows (Ternman et al., 2012) in indoor-housed environments. However, it requires a considerable amount of training to habituate the animal to wearing the equipment, and this with intensive handling, delicate and expensive devices, specialized scoring and frequent monitoring, makes PSG impractical for large research projects on cows in uncontrolled environments such as in typical group-housed farms and outdoors on pasture. No recent studies have attempted to record non-invasive PSG of sleep of cows on pasture, probably because of the difficulty in using these instruments with cows let alone in challenging and variable outdoor conditions. An ideal solution would be an alternative method or proxy for PSG, more easily applied in a variety of environments and less intensive than PSG. As cows must lie down to sleep (Ruckebusch, 1974), lying posture has been suggested as such a proxy. In calves that spend a lot more time in deep sleep stages, lying with head up and immobile and lying with the head resting on the ground or turned and resting on the flank were found to be able to estimate SWS and REM sleep time respectively (Hänninen et al., 2008). However, these same postures greatly over-estimated total sleep time in adult cows (Ternman et al., 2014) and were unable to accurately detect NREM sleep. Further methods based on accelerometers to collect movement and position data from devices on the head or neck of calves and cows (Fukasawa et al., 2018; Hokkanen et al., 2011; Klefot et al., 2016) have been developed to predict sleep. However, while these models have shown some success in detecting the tucked lying posture during which most REM sleep occurs, they overestimate total sleep time and lack the ability to distinguish differences between light and deep NREM sleep, as well as wakeful inactivity. Additionally, these methods have only been validated with postural estimates of sleep and not with PSG.

During mammalian sleep, autonomic nervous activity such as heart rate (Chouchou and Desseilles, 2014; Muzet et al., 2016; Xiao et al., 2013), respiration rate (Ebrahimi et al., 2015) and body temperature change with sleep stage. Machine learning has been used to develop wearable technology for humans such as smart watches that use heart rate and activity to predict human sleep stages and duration (Fiorillo et al., 2019). Therefore, the potential exists to use similar physiological changes to identify different sleep stages in cows. In dairy cows, respiration rate and body temperature can be recorded for long periods of time, but are difficult or require invasive internal devices (Wu et al., 2020). Heart rate (HR) and heart rate variability (HRV) recording devices are relatively inexpensive and unobtrusive to the cow and can be worn for long periods of time (Frondelius et al., 2015; Kovács et al., 2015). Methods using machine learning to predict sleep stage from HR and HRV have been developed recently for humans (Wang et al., 2019; Xiao et al., 2013), and methods combining HR with other measures such as actigraphy further increase performance for sleep stage identification (Zhang et al., 2018).

We collected HR, lying behaviour and PSG data simultaneously from two groups of cows, housed indoors and on pasture. The aim of this project was to determine if we could accurately differentiate between different stages of light and deep sleep in dairy cows using only HR and neck muscle EMG data, compared to visual scoring of the PSG, and to compare the success of two machine learning algorithms in this task.

Results and Discussion

EEG is the recognized ‘gold standard’ to determine sleep stages however, a complicated and painstaking setup is required which makes it prohibitive to use for determining sleep stages in cows. The objective of this study was to determine the efficacy of using heart rate and neck muscle activity to determine cow sleep stages using machine learning. To our knowledge, this is the first study of its kind aimed to detect cow sleep stages using only heart and neck muscle data. Using this data alone, the machine learning models developed were able to predict 82.3% of sleep stages correctly. Classification performance of the machine learning models presented in this paper is similar to Mitsukura et al. (Mitsukura et al., 2020), which proposed models to detect human sleep stages using only heart rate data. Table 1 shows the values used to compare both machine learning models. The neural network (NN) analysis produced the best overall performance and had an area under the curve (AUC) value of 92.5%. Classification accuracy was 82.3%. precision was 81.5%, recall was 82.3% and F1 score was 0.814. The prediction accuracy of the NN model is just marginally better than that of random forest (RF)which produced 82.1% classification accuracy and a slightly better AUC value of 92.6%. Both neural network and random forest algorithms show the ability to learn reasonably well from the data and discriminate well between various sleep stages.

Table 1 Overall performance of the neural network and random forest models across all sleeping stages (Average over classes) in terms of area under the curve (AUC), classification accuracy (CA), F1 score, precision, and recall (Sensitivity).

Model	AUC	CA	F1	Precision	Recall
Neural Network	92.5%	82.3%	0.814	81.5%	82.3%
Random Forest	92.6%	82.1%	0.805	81.3%	82.1%

Table 2 shows the CA and AUC of both models to predict the sleep/wakes stages individually. In terms of AUC, Awake and REM stages were the most accurately detected with a 94% and 92%

chance of scoring correctly. The models had slightly more difficulty identifying NREM sleep stages; however, AUC was remained at 90%. Figure 1 shows the ROC curves for the classification of each individual sleep stage by both NN and RF models. Classification accuracy for N3 and REM stages were above 95%, with awake and N1/2 ranging from 85-88%. Individually, N3 and light N1/2 sleep were slightly more difficult to predict according to the classification performance of various models in our dataset. As previously discussed, this could be due to errors in sleep scoring from the PSG, however NREM sleep stages are the least different from one another physiologically, so it is possible that there is a significant overlap with other sleep stages in the heart rate and neck muscle activity.

Table 2 Performance of both models (Neural Network and Random Forest) for individual sleep stages (Awake, combined light NREM sleep (N1/2), N3 (SWS) and rapid eye movement sleep (REM)) in terms of area under the receiver operator curve (AUC) and classification accuracy (CA).

Model	Awake		N1/2		N3		REM	
	AUC	CA	AUC	CA	AUC	CA	AUC	CA
Neural Network	94.7%	88.4%	90.8%	85.2%	90.2%	95.3%	92.4%	95.8%
Random Forest	94.4%	87.2%	91.1%	85.5%	90.4%	95.7%	92.3%	95.9%

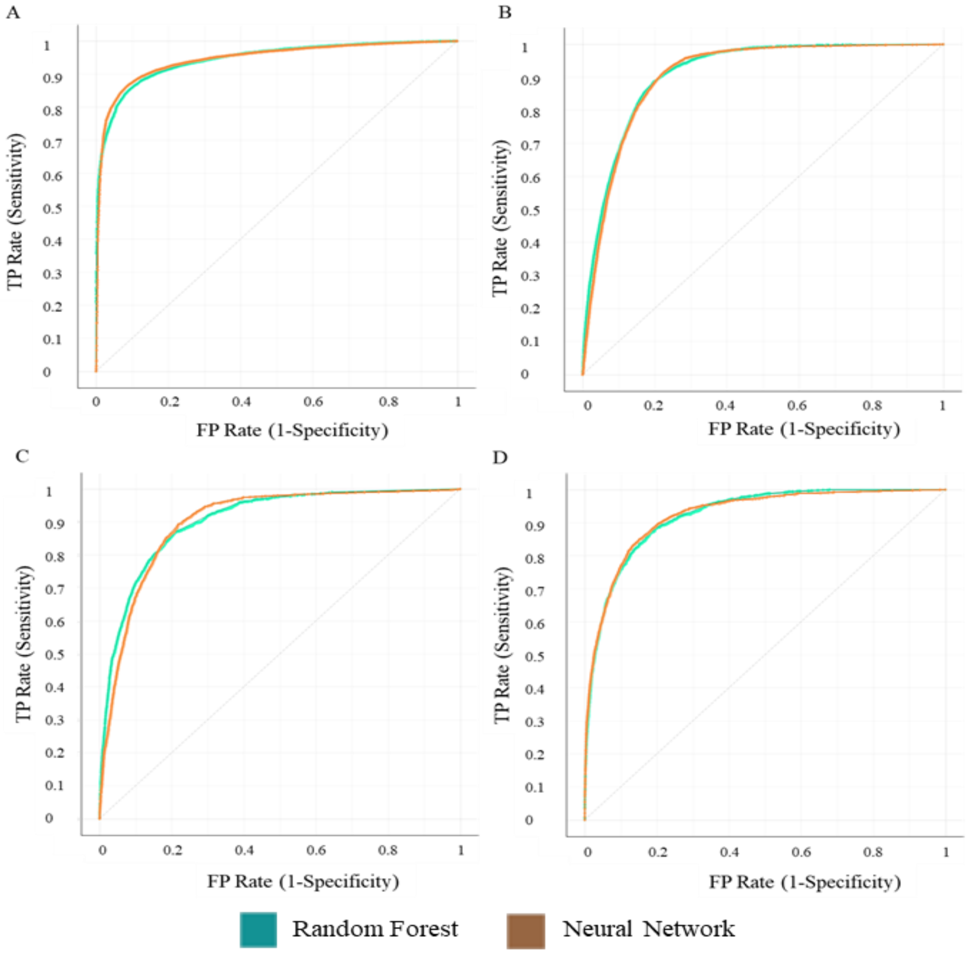


Figure*1*. ROC curves of the Neural Network and Random forest models for detection of each individual sleep stage. (a) Awake Stage, (b) Combined light sleep stages N1/2, (c) Slow wave sleep- N3 stage and (d) REM sleep stage. Figure created using Orange (version 3.26) https://orangedatamining.com/

Our methodology involved spending a significant amount of time prior to the beginning of data collection gentling and handling the cows who had previously been unused to such an amount of human contact and training them to wear unfamiliar materials and instruments. Even with these efforts, a large amount of recorded data was then unusable due to cows rubbing electrodes off on gates, water buckets or when lying or moving, unpredictable cow behaviour, or issues with electrode impedance and the devices that could only be determined after the recording. We collected a total of 23,123 useable 30 second epochs (approximately 192 hours) of PSG, HR, and activity data from a total of 12 cows in two different environments – housed indoors in the UK and on pasture in New Zealand. As there are no widely used scoring criteria for cows as there are for humans, previous work on cow sleep (Hänninen et al., 2008; Ruckebusch et al., 1970; Takeuchi et al., 1998; Ternman et al., 2012) as well as human American Association of Sleep Medicine (AASM)2018 guidelines (Iber and American Academy of Sleep Medicine, 2007) were used to define sleep stages. Previous cow PSG studies have only identified REM sleep, SWS and 'drowsing', however definitions of drowsing and implications for sleep and cow welfare are unclear (Ternman et al., 2012; Toutain and Ruckebusch, 1973). Labelling of the sleep stages based on visual analysis of the PSG traces is accepted as common practice in human sleep scoring, however, it can be somewhat subjective and there can be a degree of disagreement even between highly experienced human sleep scoring technicians using clearly defined criteria (Collop, 2002). A study of inter-rater reliability of human sleep using AASM guidelines found an overall agreement of 82.0% and Cohen's kappa = 0.76 (Danker-Hopfe et al., 2009) and a study of intra-expert scoring of spindles from light sleep found agreement of 72% with k=0.66 (Wendt et al., 2015). These kappa figures suggest high, but not perfect agreement between observers. Overall intra-observer agreement for scoring sleep/awake stages from the PSG traces in this study was 89.42%, however, N1 and N2 were the least reliable as only 32% of epochs were agreed, and 39% of N1 were re-scored as N2. Combining N1 and N2 improved agreement to 91.1%. Despite an 'almost perfect' level of intra-observer reliability (Landis and Koch, 1977), even when combining N1 and N2 stages, 8.9% of epochs were disagreed upon when re-scoring PSG. There is therefore a margin of error introduced into the model due to mistakes in scoring and labelling data from the PSG which was used as the 'ground truth' with which to train the model. However, with visual analysis there is always likely to be a degree of human error associated with the scoring.

Machine learning has also been used to classify sleep stages in animals such as mice (Brankačk et al., 2010) and rats (Crisler et al., 2008) using spectral aspects of the EEG signals, so this could be attempted in future sleep stage labelling of cow PSG data .

Cows are ruminants and must regurgitate and re-chew their food to obtain energy. Because of their strong jaw muscle movements, distinct rhythmic chewing artefacts obscure the PSG traces making accurate identification of any potential sleep stages during rumination or chewing impossible. For this reason, epochs containing rumination were excluded from the dataset and therefore the current model is only able to identify vigilance state from data when rumination is absent. Future models could be modified to predict rumination, however sleep stage estimation during this time would be impacted by the artefacts on the EMG traces.

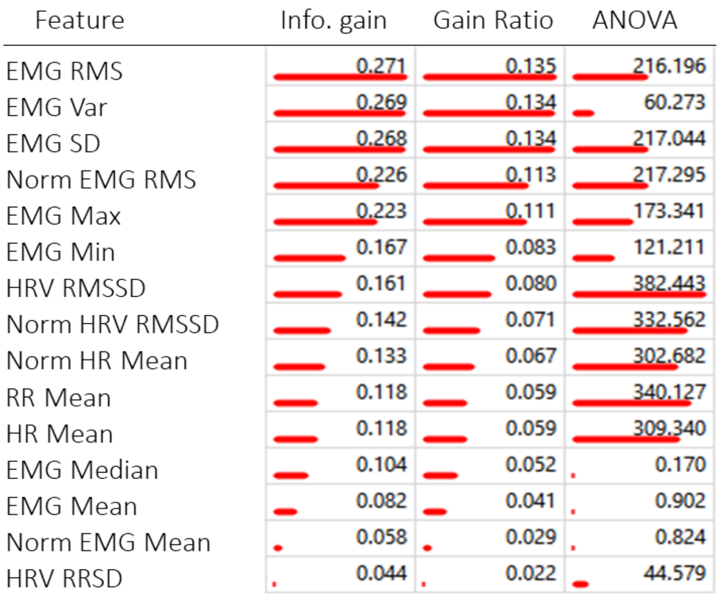
The data set was heavily weighted to the awake stage. As shown in Table 3, around 70% of the data set consisted of awake data with the other 30% consisting of sleep, and less than 5% of data points being in REM or N3 sleep stages. We made recordings during both day and night and included all recorded data of sufficient quality in the data set. Most cow sleep occurs at night-time, with small bouts of sleep during the day, and in total only about 4 hours per day is spent sleeping (Ruckebusch et al., 1970). Being so heavily weighted to the awake stage, the models had many more examples to learn from to identify Awake epochs, but far fewer examples from which to learn to identify N3 or REM sleep epochs. Balancing the dataset in terms of sleep and awake stages equally might help future models to learn better by having more examples of less common sleep stages.

Table 3 Number of data points and overall percent of data points at each sleep stage in the dataset.

Awake	16584	71.72%
N1/2	4401	19.03%
N3	1034	4.47%
REM	1104	4.77%
Total	23123	100%

We used 15 different features of the heart rate and EMG data and the machine learning models were able to learn from this and discriminate between various sleep stages. Classification models learn and perform well when there is a significant difference between features in various classes. Table 4 shows the rank of each feature calculated in terms of information gain (the expected amount of information or entropy), gain ratio (a ratio of the information gain and the attribute's intrinsic information, which reduces the bias towards multivalued features that occurs in information gain) and ANOVA (the difference between average values of the feature in different classes). The features of our dataset that were the most informative for the machine learning models were mainly the Neck EMG features (Neck RMS, Neck Variance, and Neck Standard Deviation). The highest scoring features of our dataset were the Neck EMG features (Neck RMS, Neck Variance, and Neck Standard Deviation). A reduction of muscle tone in the neck muscles is a classical indicator used for the visual identification of REM sleep from PSG data. The higher AUC and accuracy values for the prediction of REM sleep compared to other sleep stages may be due to the high rank of the neck EMG features (Table 2). Mitsukura et al. (Mitsukura et al., 2020) predominantly used frequency domain features of the HRV signal for sleep stage classification in humans, and it is possible that frequency domain features could be useful for cow sleep staging as well. However, we only used time domain features of the HRV as we were working from 30 second epochs, which is arguably too short of a window to calculate frequency domain metrics from. Frequency metrics are usually calculated for 5 minute periods, and while it could be possible to increase epoch size to 5 minutes to allow for these calculations, this would reduce the granularity and possibly result in longer epochs containing several sleep stages as some bouts of individual stages have durations of less than 2 minutes. Long epochs consisting of multiple stages could also introduce confusion into the model resulting in more misclassification.

Table 4. Ranking of features in the dataset from overall most informative to least and ranking by each calculation; Info Gain, Gain ration and ANOVA (redlines). Table produced using Orange (version 3.26) https://orangedatamining.com/



Feature	Info. gain	Gain Ratio	ANOVA
EMG RMS	0.271	0.135	216.196
EMG Var	0.269	0.134	60.273
EMG SD	0.268	0.134	217.044
Norm EMG RMS	0.226	0.113	217.295
EMG Max	0.223	0.111	173.341
EMG Min	0.167	0.083	121.211
HRV RMSSD	0.161	0.080	382.443
Norm HRV RMSSD	0.142	0.071	332.562
Norm HR Mean	0.133	0.067	302.682
RR Mean	0.118	0.059	340.127
HR Mean	0.118	0.059	309.340
EMG Median	0.104	0.052	0.170
EMG Mean	0.082	0.041	0.902
Norm EMG Mean	0.058	0.029	0.824
HRV RRSD	0.044	0.022	44.579

The classification models were developed with data from two separate groups of cows which were different in terms of breed, age, housing, and previous experience. All cows were non-lactating, but the Kiwi-cross (NZ) cows were also in mid-late pregnancy during the recording period. There were differences between the two populations in terms of average HR and the Kiwi-cross cows generally had a higher heart rate than the UK group. These differences could be due to age, size of cows and pregnancy status, but highlights the possibility of hidden batch effects within the model. More training data from different populations of cows, and cows in different stages of lactation would be beneficial to increase confidence in the classification ability of the model.

Sleep in mammals typically occurs in cycles with REM sleep following a bout of NREM however, NREM sleep can also occur on its own (Le Bon, 2020). Sleep is regulated homeostatically, but achieving a certain amount of REM sleep does not necessarily mean that a proportionate amount of NREM will also be achieved (Le Bon, 2020). In the development of the models, we considered each 30 second epoch independently, however, they are in a time series and make up bouts lasting from a few minutes to a few hours. Preceding epoch classification therefore could have an influence on the classification decision for the successive epoch. Information on typical cow sleep patterns and bout lengths could possibly aid in future models to predict sleep stages in cows.

The current model is a marked improvement over sleep staging models for cows using only accelerometers to predict NREM and REM developed in the past that were only able to predict up to 70% of sleep correctly (Klefot et al., 2016). These models also used behavioural observations to label sleep stages, which has been shown to overestimate sleep in cows (Ternman et al., 2014). Our models have been developed with sleep stages labelled using PSG rather than behavioural observations, and while not as simple as accelerometers, EMG and HR monitoring equipment are small and far easier to use with cows than a full PSG montage.

We investigated the use of non-invasively acquired EMG and HR data to predict sleep stages from light N1/2 sleep to deep N3 and REM sleep in dairy cows. While these models have been developed with a small sample size, our classification models developed with Neural Network and Random Forrest algorithms achieved similar outcomes, both with good accuracy, suggesting neck EMG and HR data could be suitable to predict sleep stage with some reliability in dairy cows. More data from cows of different breeds, ages and lactation stages would be beneficial to improve future models. We believe the use of HR and Neck EMG is promising for future identification of sleep stages in dairy cows from non-invasive physiological recording devices. This will enable future research into the effects of typical husbandry practices, transport and environment on cow sleep and the importance of sleep for cow health and welfare.

Methods

Animals and on farm management

Ethical approval for all procedures involving animals was obtained from the UK Home Office (Project Licence P204B097E), SRUC Animal Ethics Committee (Ref. ED AE 03-2018) and Ruakura Animal Ethics committee (AE 14708) prior to study onset. All methods were carried out in accordance with UK and New Zealand animal welfare guidelines and regulations and the authors have complied with the ARRIVE guidelines.

The indoor study was conducted with 6 non-pregnant, Holstein cows (average age 3.86±0.68 years) who were selected from the herd at SRUC Acrehead Farm (Dumfries, Scotland) based on farm staff knowledge of their approachable nature. When enrolled, the cows were either non-lactating or dried off according to routine farm practice prior to the study and were housed in a 20m x 5m group pen, deep bedded with straw, within the main barn and fed as per routine farm practice. A 5m x 5m test pen was located adjacent to the group pen but could be separated by a buffer zone of approximately 2m to reduce potential damage to recording equipment and disruptions to the recordings of other cows, while maintaining visual and auditory contact with the group (Fig. 2).

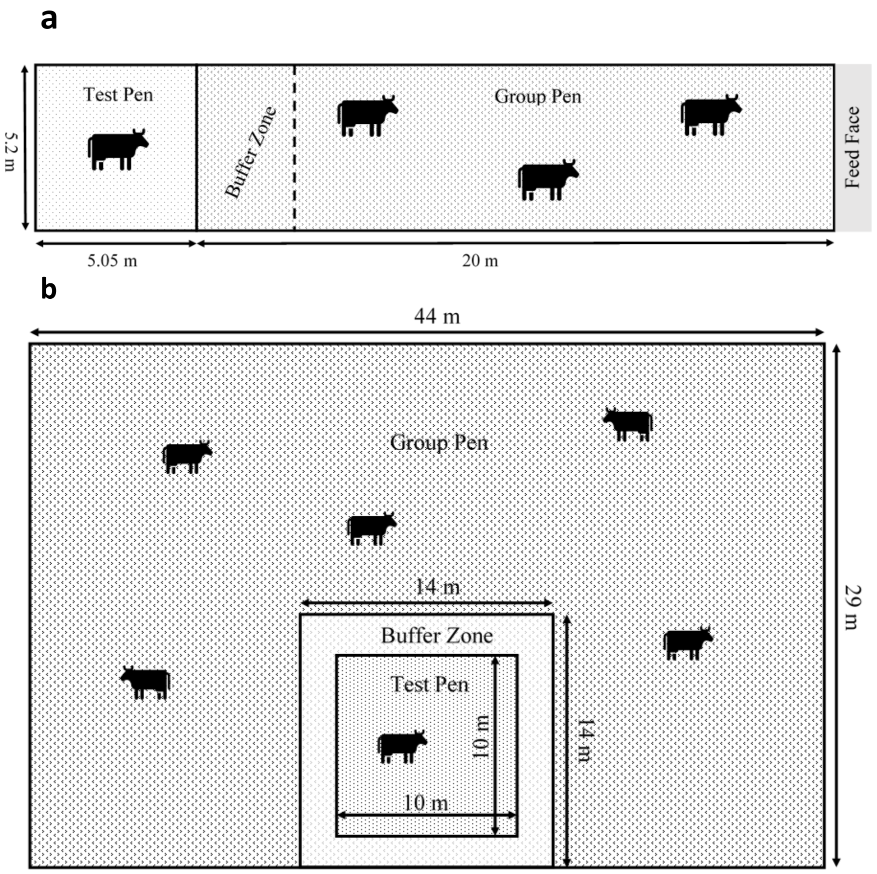


Figure 2. Diagrams of group and test pen design in the UK indoor housed study (a) and in the NZ outdoor pasture study (NZ) (b). During recordings, the test cow was moved into the test pen, when not recording, the cow was moved back into to the group pen.

The protocol was then repeated at pasture with six approachable, mid-late pregnant, non-lactating three-year-old Kiwi-cross (Friesian-Jersey) cows selected from the herd at DairyNZ Lye Farm (Newstead, NZ). These cows were managed outdoors in a large (44m x 29m) group pen created with electric fencing that could be moved around within a larger paddock as ground conditions deteriorated. A 10m x 10m test pen was created with non-live electric fencing (to reduce potential electrical noise on physiological traces) on one side of the group pen. A 2m buffer zone with live electric fencing was set up around the test pen, allowing for visual and auditory contact of the test cow with the group at all times (Fig 2). Cows were allowed to graze and were supplemented with silage *ad libitum*. All cows in both groups were trained and habituated to the recording devices and handling protocols for a minimum of 2 weeks prior to the start of data collection.

Data Collection Methods

Polysomnography

PSG was recorded using a 10 electrode montage as described in Hänninen et al.(Hänninen et al., 2008). This included 4 EEG, 2 EOG and 2 EMG electrodes as well as a ground and reference electrode attached to the head and neck of the cow (Fig. 3). Adhesive pre-gelled ECG electrodes (Natus neurology, Kanata, Canada) were used and secured to clipped and cleaned skin on the head and neck of the cow with a small amount of superglue (Loctite 454 or Loctite gel control, Henkel Corp., Dublin, Ireland). A stretchable LeMieux® or Caribu Lycra horse hood (UK: Horse Health Wessex, Woodington, UK. NZ: Caribu AU, Truganina, Australia) was modified for the cow anatomy and worn on the head and neck over top of the electrodes to keep all wires close to the skin and avoid being tangled in the test pen. After data collection was completed, all materials were removed and electrodes either came away easily or were gently removed using acetone or aqueous cream to soften the glue. Signals were sampled at 500Hz and recordings ran for 10 hours due to memory capacity of the Embletta MPR PG +ST proxy recording device (Embla, Natus Neurology, Kanata, Canada). The recording device was programmed, and data were downloaded using RemLogic 3.4.3 software (Embla Systems, Kanata, Canada). After downloading, a 50Hz mains filter was applied to all traces to remove the background noise caused from electrical wires that are present in the environment and can be picked up by the PSG device, in the UK and NZ electrical mains frequencies are both at 50Hz. EEG traces were high pass and low pass filtered at 0.3Hz and 30 Hz, EOG traces were filtered at 0.15 Hz and 20Hz and EMG at 10Hz. Traces were first inspected for quality, "good" quality recordings included those where impedance was within the acceptable range (>14Ω) and at least 2 EEG, 1 EMG and 1 EOG trace remained attached for the entire recording period. "Poor" quality recordings were not scored and occurred when impedance was too high, there was noise on the traces, many artefacts obscured the data or electrodes became detached during the recording. Traces were then scored visually in 30 second epochs into 4 stages of sleep (N1, N2, N3, REM), wakefulness (W) and rumination (RNT) by a single scorer trained in human sleep staging, according to staging criteria developed from previous work on cow sleep (Hänninen et al., 2008; Ruckebusch et al., 1970; Takeuchi et al., 1998; Ternman et al., 2012) as well as human American Association of Sleep Medicine 2018 guidelines (Iber and American Academy of Sleep Medicine, 2007).

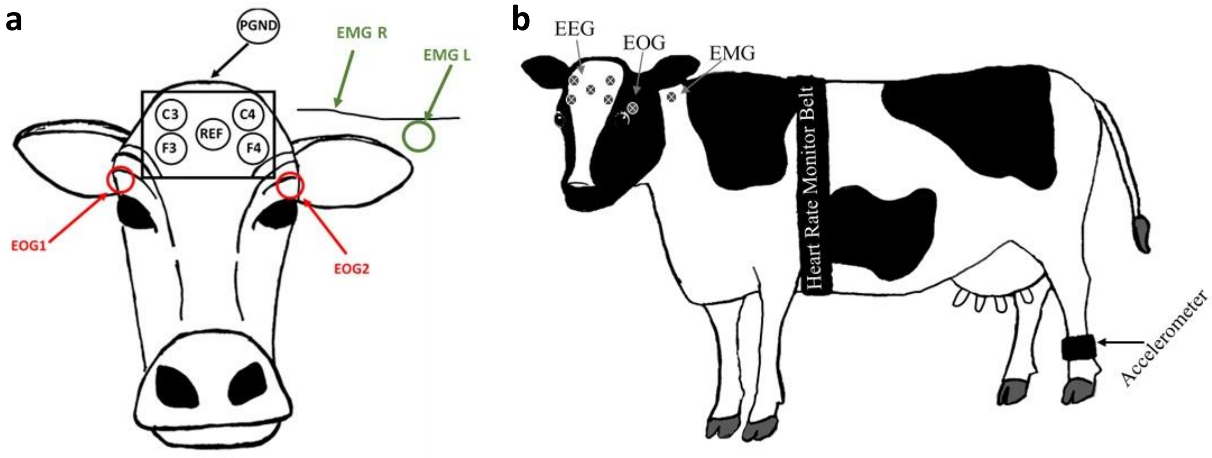


Figure 3. (a) Diagram indicating electrode placement on the head and neck of the cow for PSG data acquisition. Four EEG electrodes (C3, C4, F3 & F4) and a reference (REF) electrode were placed on the forehead. PGND- patient grounding electrode was placed behind the poll on the top of the head. Two EOG electrodes were placed beside the eyes and two EMG electrodes were placed on the mid-trapezius muscle on either side of the neck. (b) Diagram indicating placement of the heart rate monitoring girth strap, leg mounted accelerometer, and PSG electrodes on the whole cow.

Heart Rate

Heart rate (HR) and inter-beat intervals (between R peaks of the heartbeat signal: (R-R)) were recorded using a Polar equine monitoring girth strap with electrodes near the heart and the reference electrode near the shoulder (Fig 3) and logged with the Polar RS800CX Watch (Polar Electro Oy, Kempele, Finland). The time was synchronized between the watch and PSG recording devices. Ultrasound gel (Aquasonic 100 gel, Parker Laboratories, NJ, USA) was applied liberally at electrode locations. Data were downloaded using Polar Pro-Trainer 5 software (Polar Electro Oy, Finland). After downloading, the signal was filtered using Polar Pro-trainer 5 at a moderate filter power with a minimum protection zone of 6bpm. Only traces containing less than 1% identified errors were used for analysis. The filtered data were then extracted, and statistics were calculated in 30 second epochs corresponding to the timestamps of the PSG epochs. Only the time domain metrics of the heart rate variability were calculated, as the validity of frequency domain metrics in intervals smaller than the 5 minute standard are questionable (Bourdillon et al., 2017).

Lying Behaviour

Lying and standing times were recorded continuously using an accelerometer (UK; IceTags (Ice Robotics, Edinburgh, Scotland), NZ; Onset Pendant G data loggers (64k, Onset Computer Corporation, Bourne, MA) attached on the lower hind leg (Fig 3). The data were downloaded using IceManager Software (Ice Robotics, Edinburgh, Scotland) or HOBOware Pro software (Onset Corp., Pocasset, MA). Lying and standing behaviour were determined from the data-logger files in 30 second epochs corresponding to the PSG epochs.

Data pre-processing and segmentation

Neck muscle activity data was extracted from a single good quality EMG trace per recording. Statistics were calculated for each epoch, including mean, maximum (max), minimum (min), median (med), standard deviation (SD), variance (Var) and root mean square (RMS) using RemLogic software.

Mean HR, mean R-R interval, Standard deviation of RR intervals (SDRR) and Root Mean Square of Successive Differences (RMSSD) (Eqn. 1) were calculated from the exported and filtered polar heart rate data for each 30 second epoch corresponding to the PSG epochs.

$$RMSSD = \sqrt{\frac{1}{N-1}\left(\sum_{i-1}^{N-1}((RR)_{i-1} - (RR)_i)^2\right)} \qquad (1)$$

Normalized HR mean, RMSSD, EMG mean, and EMG RMS values were also calculated by dividing the data by the largest point for each individual recording as a way of removing some of the variation between cows and between recordings. All 15 parameters or ‘features’ from the HR, HRV, EMG and lying behaviour data were merged and matched with the scored sleep stage epochs using R Studio (Version 1.3.959) using time stamps and epoch numbers.

Intra-observer reliability was calculated using Cohen’s kappa in the “irr” package in R (Version 4.0.2). Overall agreement was 89.4% with k=0.83however, N1 and N2 were the least reliable as only 32% of epochs were agreed, and 39% were misidentified as N2. In exploration of the physiological data, N1 and N2 were not vastly visually different in terms of mean and variance (Fig 4), and so were combined into a new stage of light sleep named ‘N1/2’- to improve classification performance. Combination of N1 and N2 improved overall agreement to 91.1% (k=0.86). Rumination causes rhythmic chewing activity artefacts that obscure the PSG traces and make it impossible to determine brain activity and sleep stage. It is possible that cows could achieve sleep during rumination, which could create confusion and misclassification of the data, so for this reason it was removed from the data set. Dairy cows must lie down to sleep (Ruckebusch, 1974), therefore epochs determined as ‘standing’ from the accelerometer data were also removed from the data set.

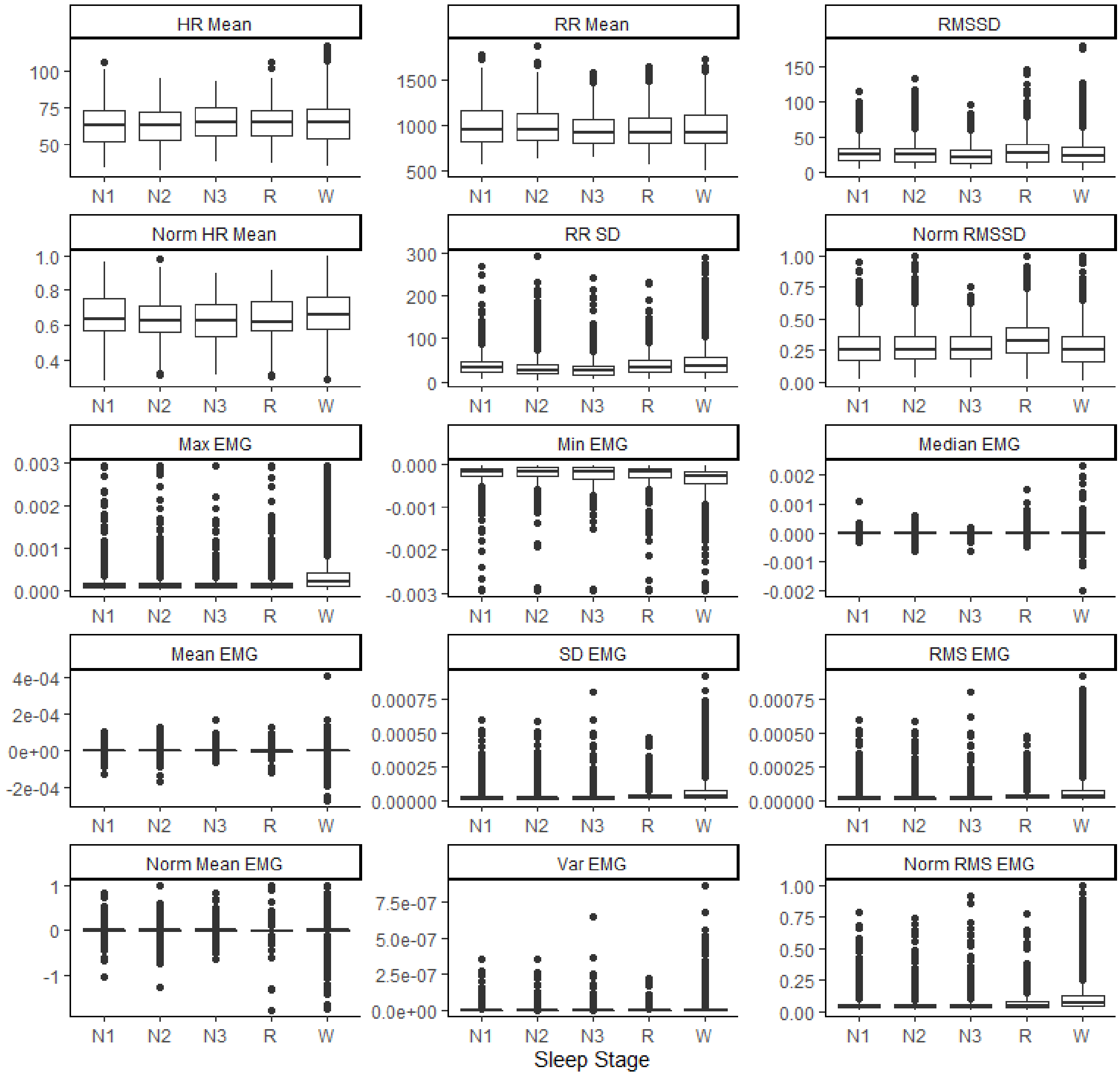


Figure 4. Box and whisker plots of each feature (Titles), with sleep stage on the x-axis and relevant units on the y-axis. The y-axis of HR mean, and Norm HR mean are expressed as beats per minute (BPM), while RMSSD, RRSD and the normalized graph of these are expressed in milliseconds. The y-axis of all EMG graphs is expressed in microvolts (µV) Figure produced in R version 4.0.2 using ggplot2 package https://cran.r-project.org/web/packages/ggplot2/index.html

From visual and exploratory analysis of the data set, there were no clear differences between sleep stages for any of the features. There were minor differences such as REM sleep tending to have a higher RMSSD than other sleep stages, and the means of W were higher for max EMG, RMS EMG and SD EMG than for the other sleep stages (Fig 4).

Altogether there were 23,120 data points labelled into 4 different sleep stages (Awake, N1/2, N3 and REM) each with corresponding data from the 15 different features (HR Mean, RR Mean etc.). Table 3 shows number of data points for each sleep stage, the awake category has the greatest number (16584) of data points, while the combination of N1 and N2 (N1/2) had 4401 data points, REM had 1104 and N3 had 1034 data points.

Machine learning method for sleep stages

To predict cow sleep stages using only heart and neck muscle data, we considered two machine learning techniques: Neural Network (Lippmann, 1987), and Random Forest (Breiman, 2001). Both the machine learning models were implemented using the open source Orange machine learning platform (Version 3.26) (Demšar et al., 2013). Stratified 10-fold cross-validation was used to train and test the models.

Architecture of the Neural Network Model:

Number of neurons in hidden layers: 500

Activation function: ReLu

Solver: Adam

Regularization: 0.0001

Maximal number of epochs/iterations: 2000

During the cross-validation process, the whole dataset was randomly split into a labelled or 'known sleep stage' data set to train the model with, the remaining data having the labels hidden and used to test the model with. For example, REM had 1104 observations, approximately 110 observations were used for testing and rest were used for training and this process was repeated 10 times for each sleep stage. The model's predictions were then compared with the actual labelled sleep stages to test and compare the models. Classification accuracy (CA) (the number of correct predictions divided by the total number of predictions), recall (sensitivity or true positive rate), precision (a measure of the model's exactness), F1 score (the balance between

Precision and Recall) and area under the curve (AUC) determined from the receiver operator curve (ROC) values from each model were used to measure the performance. The classification accuracy (Eqn. 2), precision (Eqn. 3), recall (Eqn. 4) and F1 score (Eqn. 5) were obtained from true negative (TN), false negative (FN), true positive (TP), and false positive (FP) values. This process was repeated for 10 random splits or 'folds' and classification accuracy of each machine learning technique was measured by taking the average across the 10 folds.

$$Classification\ Accuracy\ (CA) = \frac{TP+TN}{TP+TN+FP+FN} \tag{2}$$

$$Precision = \frac{TP}{TP+FP} \tag{3}$$

$$Recall = \frac{TP}{TP+FN} \tag{4}$$

$$F1\ Score = \frac{2TP}{2TP+FP+FN} \tag{5}$$

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Author Contributions

The study was conceived by LH, MH, FL, CO, JW and KS. The experiments and data collection were run by LH. Development and testing of the machine learning models were conducted by AB. The article was drafted by LH and revised by MH, AB, CO, FL, JW and KS. Drawings were prepared by LH. All authors gave final approval for publication and agree to be held accountable for this work.

Competing Interests

The authors declare no competing interests.

CHAPTER SIX

General Discussion and Conclusion

Introduction

The difficulties with assessing sleep using PSG in dairy cows became apparent in my literature review in Chapter 1. Although there is increasing interest in the study of sleep regarding cow welfare, assessing sleep accurately, affordably, and easily is a barrier for future research in this area. Much of the limited literature regarding cow sleep has focused on alternative methods for identifying sleep in dairy cows but studies validating these measures using PSG are limited. Therefore, the overall focus of this thesis was to identify and examine potential proxies for recording sleep accurately in dairy cows as compared with PSG. The first aim was to develop and describe a detailed scoring system, similar to what is used with humans, for identifying stages of light and deep sleep in dairy cows. Following on from the development of these methods, I aimed to determine if lying postures, and particularly the position of the cow's head while lying could predict these detailed sleep stages compared with the PSG scoring system. I then aimed to determine if cow's HR and HRV changed with sleep stage, and if this could be of potential use for sleep stage prediction. Finally, I aimed to determine if multiple physiological signals obtained from non-invasive surface attached devices could be used as an alternative to predict sleep stages in dairy cows as accurately as PSG. In this chapter, I will discuss the findings of the main works of the thesis and the wider implications of the results. I will also discuss the limitations and consider areas for future research.

General Limitations

The experiments carried out during this thesis were limited by very small sample sizes: 6 cows in Scotland and 6 cows in New Zealand. The small sample size was necessary as it was very time, and labour, intensive to train and record data from the cows, however, this small sample size does make it difficult to extrapolate the results to dairy cows in general. Although I expect that major patterns of physiological changes during sleep should persist among all cows, it is possible that differences in the patterns of physiological changes with sleep stage can occur between breeds, ages, physiological status and management systems as seen between cows in the Scottish and New Zealand systems.

A main strength of the studies included in this thesis is the replication of the experiments with indoor housed cows in Scotland and outdoor pasture managed cows in New Zealand. We proved it is possible to adapt PSG methods to record free moving cows in outdoor environments despite changing weather conditions. Importantly, we found that the PSG, HR and HRV showed similar patterns with sleep stages for cows in both environments. However, the use of two environments was also a limitation and made it difficult to be able to determine whether the cause of the observed differences in the sleep behaviour between studies was caused by the housing environment, weather conditions, or from differences in breed, physiological status, or country between the cows.

Sleep scoring methods

In chapter 2, I described a five-stage scoring system developed for dairy cow sleep based on human scoring guidelines and visual analysis of PSG traces. Using the guidelines resulted in good intra-observer reliability, although reliability was lower between lighter N1 and N2 sleep stages. This has also been found to be an issue in human sleep scoring (Suzuki et al., 2019) and it has been found that there is only moderate and sometimes poor agreement between scoring technologists in different scoring labs (Collop, 2002; Danker-Hopfe et al., 2004), and poor or moderate agreement in intra-observer agreement has been shown as well (Suzuki et al., 2019). Inaccurate scoring of the PSG has impacts on the results of the other chapters as it is used for scoring and is designated as the gold standard reference for sleep stage identification. This could have implications for the other methods described in chapters 3-5, where for example, instances of mis-scoring between N1 and N2 could have affected the accuracy of the proxy method to identify the correct stage.

The outputs or traces from the PSG are often scored visually, as there can be individual differences in brain activity between subjects, and human scorers using standardized criteria can often be more adaptable to these differences than current computer algorithms (Silber et al., 2007). However, computer algorithms have been developed to identify sleep stages in humans (Faust et al., 2019) and in other animals such as rats and mice (Allocca et al., 2019; Lampert et al., 2015). During this study we attempted to run the automatic human sleep staging algorithm included with the RemLogic software on the cow PSG but found that most epochs were obviously scored incorrectly. Using the built-in scoring algorithm, epochs clearly containing rumination were scored as N3, likely due to the slow wave artefact from the chewing activity. Because of the rumination artefact, using previously developed sleep staging algorithms for other animals may not be useful for scoring sleep in ruminants.

The methods and devices required to record PSG are time consuming and difficult to use, and scoring PSG manually is also time and labour intensive, and human scoring can be subjective. Thus, investigation into the differences in the characteristic activity and power spectra of the EEG or other PSG traces could be useful to develop more objective automatic or semi-automatic scoring systems for cow sleep in the future.

This scoring system is the first to score lighter stages of NREM sleep in dairy cows. Previous criteria for scoring sleep in dairy cows described REM sleep, Slow Wave Sleep and 'drowsing' (Hänninen et al., 2008; Ruckebusch et al., 1970; Ternman et al., 2012). As mentioned earlier in this thesis, Ruckebusch (1972) described drowsing as a stage of stable wakefulness, indicating that he believed the cows to awake but inactive during this time. More recent work in cows that have also scored drowsing according to the criteria described by Ruckebush noted the occurrence of sleep spindles and k-complexed during this stage (Ternman et al., 2012). Sleep spindles, short 1 second or so bursts of 11-16 Hz activity (Iber and American Academy of Sleep Medicine, 2007), are characteristic of human N2 sleep and were also observed on the cow PSG during drowsing epochs (Ternman et al., 2012). Thus, drowsing may be better classified as a stage of light sleep rather than wakefulness. Sleep spindles can be difficult to identify reliably. A study of inter-expert and intra-expert agreement for human sleep spindle scoring found weak to moderate agreement in the ability to identify sleep spindles correctly (Wendt et al., 2015). The difficulty in reliably identifying sleep spindles could be a source of disagreement in scoring between N1 and N2, as epochs of N2 stage sleep with no sleep spindles or K-complexes could appear to be similar to N1. Sleep spindles have been described in sheep and can be detected using an automatic algorithm (Schneider et al., 2020). Using an algorithm to detect sleep spindles or other patterns of the cow PSG could also be a way to improve sleep stage identification, particularly for the lighter N1 and N2 stages.

The description of the 5 stages of sleep in this study are based solely on their visual similarity to well described human sleep stages. The scoring system I developed for the cow PSG was influenced by the pre-conceived scoring systems developed for humans, splitting NREM into 3 stages, however, these divisions may not necessarily be required for cows. Human sleep has been differentiated into these stages to categorize different brain patterns as well as depths of sleep. Sleep depth can be determined from auditory arousal thresholds (Busby et al., 1994; Pilon et al., 2012) and generally, it takes less auditory stimulation to wake someone from light sleep than it is to wake them from a deep sleep. Verification of the classification of sleep stages and suitability of splitting NREM into multiple stages in dairy cows could be accomplished by investigating the auditory arousal thresholds compared to PSG.

Using unsupervised machine learning methods could also be useful way to identify sleep stages from the PSG data alone, without the bias associated with pre-conceived scoring frameworks. Unsupervised machine learning methods use unlabelled data as opposed to supervised machine learning that requires human input to classify the data into categories, and are able to identify hidden patterns in the data without human direction (Sathya and Abraham, 2013). Clustering algorithms, that group data into more homogenous clusters could be an option to classify the raw data into similar stages (Jafari-Marandi, 2021).

Cows must ruminate to break down the fibre in their diets and can spend large amounts of time per day chewing (Beauchemin, 1991), however it is unclear if a certain level of cognitive arousal is required for this behaviour to occur. In this thesis, rumination was scored as a distinct stage on the PSG traces and removed from the dataset completely in analysis of the HR and development of machine learning models because the actual brain activity during this time could not be determined accurately. A filter has been specifically created to attempt to remove the rhythmic chewing artefact from the EEG traces in dairy cows using the EMG trace (Pastell et al., 2012). While the filter appears to work well in being able to remove a large amount of the error in the signal, the corrected trace can appear to contain spindles, which may still cause confusion during scoring. I observed that the cows would go from rumination straight into REM sleep (Appendix A). This type of sudden cataplexy or loss of muscle tone, and the presence of REM-like EEG traces are criteria used to identify narcolepsy in animal models (Toth and Bhargava, 2013). However, rather than being a type of narcoleptic transition, it may be more likely that cows are able to sleep during rumination and transition directly to REM from this rumination/sleep state. When ruminating, cows need to pause chewing to swallow and regurgitate the cud. This gives a small 3-4 second window without chewing to observe the EEG unobscured by rumination artefact. Such a short window is not typically used in human sleep scoring, and may be misleading for visual analysis (Schulz, 2008), but could be a method to identify sleep stages during rumination in cows. Cows have been found to ruminate for around 6-8 hours per day (Stone et al., 2017) and rumination is often correlated with lying behaviour (Schirmann et al., 2012; Stone et al., 2017), therefore cows may be able to achieve more sleep than previously thought, and particularly more light or NREM sleep.

Lying postures

We found that lying postures could not be used to identify sleep stages that were identified using PSG as the gold standard in chapter 3. This was similar to results found by Ternman et al. (2014), who found that lying postures could not be used to identify REM and NREM sleep in dairy cows. We found that more specifically defined head postures were also not useful to identify stages of N1 and N2 sleep as hypothesised. REM sleep did occur in a head resting or tucked posture most of the time, and almost all of the time in the New Zealand study; however, using only these postures would overestimate actual REM sleep time substantially. Moreover, using the tucked or “sleep posture” to estimate total sleep time (Fukasawa et al., 2019, 2018) would be an underestimation of total sleep time and overestimation of REM. There is as yet no evidence to suggest that REM sleep is expressed proportionately to the amount of other sleep stages that a cow will achieve. However, recently, Ternman et al. (2019) found that REM sleep time changed with the lactation cycle, and also showed that other sleep stages, such as NREM and drowsing also changed similarly. More research is needed to determine if sleep stages are exhibited proportionally in dairy cows and if so, if total sleep time could potentially be estimated through the identification of only one stage.

In a study to investigate the use of accelerometers on a halter to study sleep in cows, Klefot et al. (2016) used in-person monitoring of the cows’ lying postures, head positions as well as eye closure and opening to identify stages of NREM and REM sleep and wakefulness. We attempted to record eye opening and closure during behavioural observations of the cows, but even from the four corners of the test pen, and infrared cameras, were not always able to accurately determine when the eyes were open or closed. It could be possible for cows to be asleep with eyes open (McNab, 2005), or alternatively, to be awake with eyes shut. Future investigation of this behaviour could be conducted using PSG methods for clarification.

Heart rate

In chapter 4, HR was recorded simultaneously with PSG and the HR and HRV measures were found to change between sleep stages in dairy cows. The differences between stages followed similar patterns between the Scottish and New Zealand cows despite large differences in the mean heart rate between the groups. Again, repetition of the experiments with different breeds, ages, physiological status and sizes of cows would be beneficial to be able to apply the use of HR and HRV as proxy measures of sleep to all types of cows.

The heart rate signals recorded from the cows contained artefacts. Artefacts can be physiological in nature, originating for example from ectopic (extra) heart beats, or from technical issues with the HR recording devices (Peltola, 2012). PolarPro Trainer 5 software was used for correction of the HR data in chapters 4 and 5 with moderate filter power, which detected and corrected errors based on deviation from the HR curve, and a minimum protection zone of 6 beats, above which the filter power would not adjust the HR reading. Only recordings where the HR correction methods corrected just a small proportion of beats over the entire recording were included for analysis. Different editing methods such as deletion or interpolation can be used for correction of HR signals. They are important for the accurate reflection of HRV measures, however these filtering methods also have an effect on the results (Peltola, 2012). Additionally, short term HRV analyses are more sensitive to artefacts, and editing and the use of 30 second epochs in the studies of this thesis could have affected the accuracy of the HRV metrics calculated. In future, analysis of the HR data using different filtering methods, or longer epoch lengths, could improve accuracy of the HRV analysis for cow sleep.

Some problems were encountered when recording the HR signals in the cows, mainly issues with artefacts or signal loss due to technical issues with the recording devices or degradation of the signal during long-term recording. Polar HR monitors are often identified as being the most accurate and are suitable for long term recordings up to and over 24 hours in length (Hinde et al., 2021). The artefacts encountered may have been caused by movement of the electrode belt which could have been exacerbated by free movement of the cows in the test pen or in the paddock, or from the ultrasound gel on the electrode pads drying out. Tightening the belts and the girth may be a way to reduce the likelihood of movement of the belt and therefore the number of artefacts in the data but may be uncomfortable for the cows. Further investigation regarding the accuracy of long-term heart rate recording methods would be beneficial, particularly if attempting to use HR signals in sleep assessment, as long-term monitoring for 24 hours or at least over-night is required.

Over the past few decades, other methods of heart rate monitoring, specifically optical heart rate monitoring technology, have improved significantly. Photoplethysmography (PPG) is an optical technique that uses changes in the refractory rate to detect pulse rate from optical changes of blood circulation in the skin (Allen, 2007). Many human smart watches use PPG and machine learning algorithms to identify heart rate for humans, however there is some concern regarding the use of PPG for accurate HRV detection (Hoog Antink et al., 2021). PPG is used in veterinary practice to monitor the pulse oxygenation of the animals, usually from a clip on the tongue during surgical procedures, but more recently, a study found good comparison of tongue pulse oximetry with sensors placed on the tail of dogs and around a cat's paw (Cugmas et al., 2019). Deep learning methods have been developed to predict sleep stages from PPG signals with moderate accuracy compared with PSG for humans (Korkalainen et al., 2020). PPG could be a potential method to measure HR in dairy cows and may also be useful to include in future models to predict sleep stage.

Machine learning

Applying machine learning methods, I developed a method to predict sleep stage as identified from the PSG traces using a combination of non-invasive HR measures and surface EMG. The two supervised machine learning algorithms performed similarly and were both able to classify the data well and predict the correct sleep stages. Supervised machine learning works by using the already classified or scored data as an example with which to teach the model. As discussed earlier, using unsupervised learning, and allowing the algorithms to identify the most likely or natural groupings of the data could be a method for future refinement of the models.

The models were built and tested with a data set with standing and rumination removed making it easier for the model to learn to identify the sleep stages correctly, however this is a somewhat ‘un-natural’ data set. Adding the standing and rumination data in future models would be beneficial for real life scenarios in future applications. Adding in these sorts of real life, messy data sets could enable future development of these models to be able to predict the sleep of cows in real-time. Live HR and HRV can be accurately detected with a smartphone app rather than having to log the data with a Polar watch for dairy cows (Wierig et al., 2018) and wireless surface EMG devices have been developed for human activity monitoring (Biagetti et al., 2018). Further research into the use of these devices with dairy cows, and in outdoor free ranging environments could be a way to identify particular sleep stages and to investigate the effects of selective sleep deprivation, to identify the importance of individual sleep stages for cows.

Further improvements to the current predictive model could also be made through evaluation and learning of the patterns and probabilities of sleep stage transitions. Appendix A contains a transition matrix of the probabilities of the epoch of a sleep stage being followed by each of the other sleep stages. In general, the cows followed similar patterns to humans (Carley and Farabi, 2016), moving from awake to lighter sleep (N1&N2) into N3 and REM followed by arousal back to awake. Using the transition probabilities, or more complex computer algorithms such as long term/short term memory functions could improve the predictive ability of the model by cutting down on the likely possible epoch classification based on experience from previous epochs (Radha et al., 2019).

HR and HRV were included in the predictive models for sleep stage, however the model could only learn from the discrete data points to have input on the classification decision. Because of this, HR and HRV features were not as important to the model as the EMG data were. However, in chapter 4 we found the HR and HRV features did differ between sleep stages, and often there was a significant difference between the sleep stages. These cardiac variables were particularly effective at distinguishing REM and N3 sleep from awake. Therefore, by using the typical patterns of differences in the sleep stages using identified periods of wakefulness as a baseline, HR and HRV features could become much more useful in future machine learning models.

Potential uses of non-invasive methods for assessing cow sleep

The development of these method for detecting sleep could facilitate future research in dairy cow welfare and could assist in the investigation of many questions regarding sleep, cow behaviour and welfare. For example, how short term or chronic sleep loss events, uncomfortable surfaces, restrictive lying areas, and high stocking densities affect the abilities of cows to get sufficient sleep.

The PSG recording length during the studies in this thesis was limited to 10 hours due to memory of recording devices and we chose to start recordings at 7pm and 7am ending at 5am and 5pm respectively, based on human-centred time schedules. Upon analysing the data, we found that when the recordings stopped at 5am the cows were often right in the middle of a bout of sleep. This observation raises the subject of the impact of the timing of farm processes and specifically milking frequency on cow sleep. On farms that milk twice or three times a day, early morning milking times can start at 4am or even earlier. In a study of milking frequency of cows in Israel where milking three times a day is common, cows were milked at around 1am, 1pm and 8pm (Moallem et al., 2019). A study of dairy cows using automatic milking, or robotic milking in Australia identified three main groups of cows, who showed shifts in the mean number of milking events per hour (John et al., 2019). This could indicate that individual cows may have different chronotypes or sleep timing preferences. Some cows may prefer to sleep-in, and some may be early risers, therefore developing an understanding of typical sleep patterns and timing of sleep could be beneficial for improving cow sleep and cow welfare on farms.

Pasture based dairy cows in New Zealand may occasionally graze in paddocks that are a good distance away from the milking shed, meaning that they may need to walk long distances to milking in addition to their daily movement activity while grazing. These cows are therefore likely to have much more movement opportunities than cows housed indoors in free stall or even tie stall environment (Shepley et al., 2020). While movement opportunity may not be exactly comparable with human exercise, it can indicate more opportunities for physical activity. In humans, a positive relationship between perceived sleep quality and physical activity has been reported, particularly in older adults (Christie et al., 2016). The methods developed during this thesis could be used in the future to investigate the effects of movement opportunity on sleep and sleep architecture in dairy cows.

Intensive winter grazing on crop or paddock is common in the South Island of New Zealand but grazing on crop is also becoming increasingly common in the North Island. Cows are held on restricted areas of the pasture or crop (break/strip) at a high stocking density, new breaks are given daily or when needed and they may also be given supplementary silage or hay. This is done to avoid pugging damage to the paddocks as the soil can be affected by seasonal increases in rainfall and shorter day-lengths affecting re-growth (Houlbrooke et al., 2009). However, due to increases in rainfall, and high stocking density, surfaces can quickly become wet and muddy, these factors can cause reductions in lying time, rumination time and choice of lying site which are all significant welfare concerns for cows. Dairy cows must lie down to sleep, therefore conditions that significantly affect lying times may also influence a cow’s ability to achieve enough sleep. The NZ dairy welfare code recommends cows should be able to lie and rest comfortably for 10-12 hours per day (Ministry for Primary Industries, 2019). Studies with NZ dairy cows have found significant reduction of lying time during wet days on crop paddock (5hrs/ day, Al-Marashdeh et al., 2019) and in simulated winter conditions on grass pasture (4hrs/day, Tucker et al., 2007). In a simulated mud experiment, lying times were reduced proportionally with increasing moisture content, white blood cell counts were negatively affected and cows spent more time lying on concrete, rather than lie in very muddy conditions (Chen et al., 2017). Recently, Schütz et al. (2019) found that moisture content rather than 'dirtiness' was the main element affecting a cow's decision to lie down. Cows also change their lying postures on wet surfaces to minimize body surfaces touching the ground and spend significantly less time in tucked or head resting positions (Schütz et al., 2019; Tucker et al., 2007). Temporarily removing or 'standing off' the cows from the paddock for a portion of the day onto a 'stand-off pad', sacrifice paddock or occasionally a farm laneway, are some of the management practices used to reduce the impact of the cows on the paddocks in winter. However, uncovered standoff pads are also subject to the winter elements and without refreshing the bedding surfaces regularly, can become wet and muddy leading to significant and serious reductions in cow lying time (O'Connor et al., 2019). The methods developed during this thesis could be used to investigate the impact of winter grazing practices, muddy surfaces, stocking density as well as stand-off pads of the sleep and sleep architecture of dairy cows to inform winter management practices on farm.

Additionally, the methods developed during this thesis could also be applied to other animals. Long term monitoring of animal sleep has often necessitated implanted electrodes (Malungo et al., 2021; Perentos et al., 2017; Ruckebusch et al., 1970). The use of surface electrodes to record HR and EMG would be a way to refine these methods, improving animal welfare during sleep research.

Final Conclusion

Sleep is important and needs to be considered for animal welfare. There are many factors in the environment and management of dairy cows that are likely to have an impact on sleep and methods to study sleep more easily and reliably are required to include sleep in future animal welfare research. Overall, the results of my thesis have expanded the understanding of sleep physiology in dairy cows. The studies presented in this thesis have provided an expanded framework for the visual assessment of sleep from PSG and have reinforced the need for alternate ways of identifying sleep other than PSG and behavioural methods. I have demonstrated that methods of identifying sleep from heart rate and muscle activity can be as accurate as verified using PSG, with accuracies in the range shown in similar research with humans. The results of my thesis are a significant advancement for the future of sleep research in cows, however further research is required to validate these results with larger sample sizes, different environments, and different cows.

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APPENDIX A

Additional General Results

In this section I have included results not presented in the main text, including information about the amount of data collected, the breakdown of sleep stages into bouts and bout lengths as well as stage transitions.

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Data Recorded and Quality

Overall, 32 recordings were made with the Scottish cows, and 35 with the NZ cows. Table 1 shows the total amount of time recorded and the break down of this time into good, OK and bad quality data. Good quality data included recordings where at least one trace of each of the PSG signals had good impedance and the HR signal was also good based on the criteria described in chapter 4. OK quality data included recordings where one of the PSG traces was missing or had poor impedance, the recording cut out halfway, or the heart rate cut out halfway. Bad quality data included recordings where several of the PSG traces were missing or had poor impedance or the HR signal was poor or missing.

Table 2 shows the number of recordings made with each cow as well as the number and percent of good quality recordings made with each cow. On average we made 5.6 recordings with each cow, but only 55% of the recordings made were successful, however this ranged from 0-100% depending on the cow.

Only the good quality data, and the data classified as OK due to recordings cutting out halfway but that otherwise contained good quality data were used for scoring and included in the results in the main body of this thesis. Table 3 shows the breakdown of the total amount of the scored data by country and time of recording. Daytime recordings ran from 7am to 5pm and night-time recordings from 7pm to 5am. Table 3 also shows the breakdown of the scored data into total sleep time, total time awake and total time ruminating by study and time of day. Overall, 377.7 hours of data were scored, almost half from recordings made in the daytime and half from overnight recordings. Over all the recordings, 17.64% of the time was scored as sleeping (N1, N2, N3 & REM), 48.9% of the time was scored as awake, and 33.7% was scored as ruminating. In both the Scottish and NZ data alone, about 30-35% of the data was scored as ruminating. In Scotland, about 25% of the data was scored as sleeping and 43% was awake. In NZ however, only around 12% of the data was scored as sleeping, and 52% was scored as awake. In both groups, about 70% of the sleep occurred during night-time recordings, and 30% occurred during the day, and rumination followed a similar pattern. These results could indicate that the Scottish cows slept more than the NZ cows, but as we were only able to record for 10 hours at a time, and had a large amount of unusable data, we were unable to interpret the amount of sleep over a 24 hour period.

Table 1 *Number of recordings made, recording lengths, and recording quality for the Scotland and New Zealand cows.*

# Scotland Recordings:		**32**	**# New Zealand Recordings:**		**35**
Total Hours Recorded:	241:11:55	Percent of Total:	**Total Hours Recorded:**	338:48:00	Percent of Total:
Good Quality Total:	159:36:27	66.17	**Good Quality Total:**	208:51:00	61.64
OK Quality Total:	50:56:34	21.12	**OK Quality Total:**	30:00:00	8.85
Bad Quality Total:	15:39:57	6.49	**Bad Quality Total:**	99:57:00	29.50
Good/OK Total:	210:33:01	87.29	**Good/OK Total:**	238:51:00	70.50

Table 2 *Total number of recordings and good quality recordings made with each cow*

Country	**Cow ID**	**Name**	**Total # Recordings**	**# Good Recordings**	**% Good Recordings**
NZ	5393	Ellie	5	3	60%
NZ	5298	Millie	7	2	29%
NZ	5344	Bobby Brown	5	3	60%
NZ	5762	Annie	7	4	57%
NZ	5037	Tango	5	4	80%
NZ	5035	Mango	6	6	100%
Scot	2481	Sassy	4	1	25%
Scot	2457	Merida	2	0	0%
Scot	404	Page	7	3	43%
Scot	369	Maisel	9	7	78%
Scot	367	Dina	6	5	83%
Scot	337	Monster	4	2	50%

Table 3 *Total hours of data scored from UK and NZ cohorts, total time from scored data scored as sleeping, awake and rumination, during the day and night by cohort.*

	Scot	**NZ**	**Total**
Overall total Scored Data	**167.06**	**210.64**	**377.7**
Day	68.84 (41.21%)	109.84 (52.25%)	178.68 (47.31%)
Night	98.22 (58.79%)	100.8 (47.85%)	199.02 (52.69%)
Total Sleep Time *(N1, N2, N3 & REM)*	**41.73 (24.97%)**	**24.92 (11.83%)**	**66.65 (17.64%)**
Day	12.97 (31.08%)	7.51 (30.14%)	20.48 (30.73%)
Night	28.77 (68.94%)	17.41 (69.86%)	46.18 (69.29%)
Total Wake Time	**72.29 (43.27%)**	**111.6 (52.98%)**	**183.89 (48.86%)**
Day	42.3 (58.51%)	80.28 (71.94%)	122.58 (66.66%)
Night	29.99 (41.49%)	31.33 (28.07%)	61.32 (33.35%)
Total Rumination Time	**53.03 (31.71%)**	**74.13 (35.19%)**	**127.16 (33.66%)**
Day	13.58 (25.61%)	22.06 (29.76%)	35.63 (28.02%)
Night	39.46 (74.41%)	52.07 (70.24%)	91.53 (71.98%)

Sleep Stage Bout Lengths

Bout lengths were calculated by adding successive epochs until a stage shift was identified. Figure 2 indicates the average bout lengths in each sleep stage, awake and rumination for both study countries separated by recording time. Table 4 also shows these results, as well as the total number of minutes, and percent of total time in each sleep stage by country and time of day. Bout lengths in N1 were very short and lasted on average about 1.4±1 minute in both groups in both day and night recordings. N2 bouts were also quite short, lasting on average 2.6±2.3 minutes in both daytime and night-time recordings for both groups. On average N3 bouts were 3.1±2.1 minutes long, however N3 bouts were shorter in the daytime (2.5±1.4 min) than at night (3.4±2.3 min). REM sleep bouts were on average 4±2.4 minutes long, however, were also much shorter during the daytime (2.6±1.7 min) than at night (4.3±2.4 min). Very little REM sleep was scored during the daytime, only 83 minutes of REM sleep were scored during the daytime. Bouts of rumination lasted on average 31.6±19 minutes and were shorter during the daytime than at night. Awake bouts were quite variable in length, however the NZ cows had longer awake bouts, particularly during the daytime.

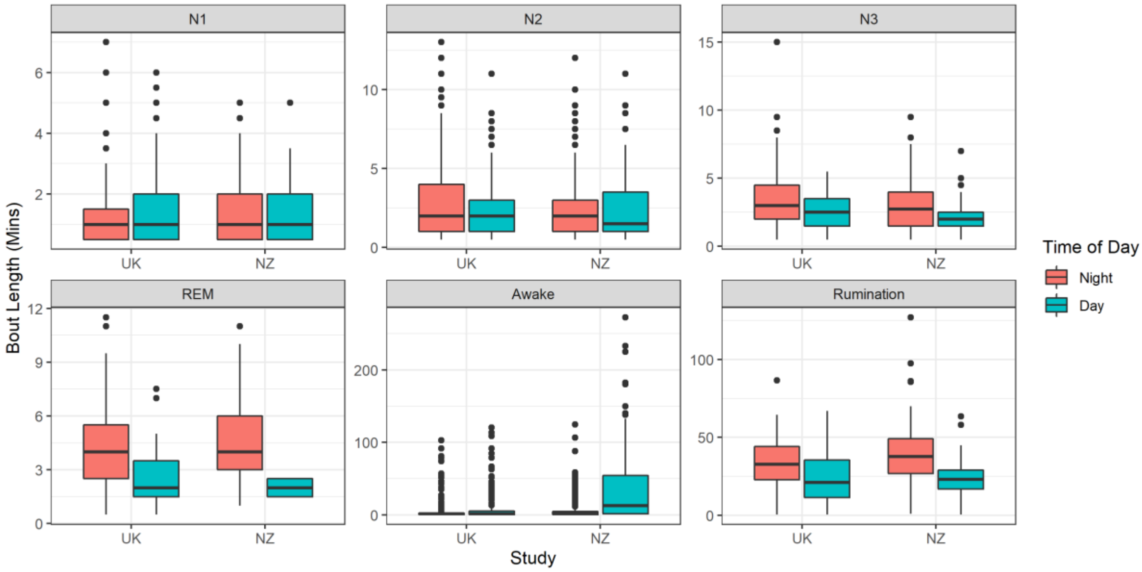

Figure 1 *Bout lengths (min) in each sleep stage (N1-N3, REM), wakefulness and rumination, by cohort group in daytime and night recordings.*

Table 4 *Total time and bout lengths by sleep stage, time of day and cohort group.*

Sleep stage, Time of Day, Study	***Total Minutes Recorded (% of total)***	***Average Bout Length (min) ± StdDev***
N1	**1036 (4.8%)**	**1.4±1**
Day	*356 (34.4%)*	*1.5±1.1*
NZ	100.5 (28.2%)	1.4±1
Scot	255.5 (71.8%)	1.5±1.2
Night	*680 (65.6%)*	*1.3±1*
NZ	255 (37.5%)	1.4±1
Scot	425 (62.5%)	1.3±0.9
N2	**1710 (7.9%)**	**2.6±2.3**
Day	*469.5 (27.5%)*	*2.4±2*
NZ	156.5 (33.3%)	2.6±2.4
Scot	313 (66.7%)	2.4±1.8
Night	*1240.5 (72.5%)*	*2.7±2.4*
NZ	412 (33.2%)	2.6±2.2
Scot	828.5 (66.8%)	2.8±2.4
N3	**597 (2.8%)**	**3.1±2.1**
Day	*129.5 (21.7%)*	*2.5±1.4*
NZ	55 (42.5%)	2.4±1.6
Scot	74.5 (57.5%)	2.6±1.3
Night	*467.5 (78.3%)*	*3.4±2.3*
NZ	196 (41.9%)	3.2±2.1
Scot	271.5 (58.1%)	3.5±2.5
REM	**745.5 (3.5%)**	**4±2.4**
Day	*83 (11.1%)*	*2.6±1.7*
NZ	10 (12.0%)	2±0.5
Scot	73 (88.0%)	2.7±1.8
Night	*662.5 (88.9%)*	*4.3±2.4*
NZ	300.5(45.4%)	4.6±2.4
Scot	362 (54.6%)	4.1±2.3
Awake	**10105.5 (46.9%)**	**11±27.1**
Day	*6456.5 (63.9%)*	*21.1±40.5*
NZ	4455.5 (69.0%)	39.8±55.7
Scot	2001 (31.0%)	10.3±22
Night	*3649 (36.1%)*	*6±14.2*
NZ	2012.5 (55.2%)	8.1±16.2
Scot	1636.5 (44.8%)	4.5±12.4
Rumination	**7342.5 (34.1%)**	**31.6±19**
Day	*1988.5 (27.1%)*	*24.3±14.7*
NZ	1195.5 (60.1%)	24.4±12.2
Scot	793 (39.9%)	24±18.1
Night	*5354 (72.9%)*	*35.7±19.9*
NZ	2989 (55.8%)	39.3±21.7

Scot	2365 (44.2%)	32±17.3

Sleep Stage Transitions

Sleep stage bout transition matrices were calculated in R version 4.0.5 (R Core Team, 2021) using the ‘markovchain’ (Spedicato, 2017)and ‘msm’ (Jackson, 2011) packages. Figure 2 shows the proportion of bout transitions for all sleep stages (N1, N2, N3 & REM), awake (W), rumination (RNT) as well as epoch and bouts scored as unknown (U). The majority of W bouts were followed by a bout of N2 (66%), or rumination (18%). N1 sleep was often followed by a bout of N2 62% of the time or back to W in 30% of cases. In 38% of cases, N2 was followed immediately by a W bout, but 27% of the time proceeded into N3. Almost 40% of N3 bouts were also followed by a W bout, 31% of the time they returned to N2, and 17% of the time were followed immediately by REM sleep. Ninety percent of the time, REM sleep was immediately followed by W. About 70% of bouts of rumination were followed by W bouts, but approximately 20% were followed by light sleep (N1 &N2). Rumination was proceeded by REM sleep 4% of the time. Table 5 shows the actual number of bout transitions to and from each sleep stage. Figure 3 and Table6 show the transitions for only the night-time recordings. Figure 4 and Table 7 show the transitions for the daytime recordings.

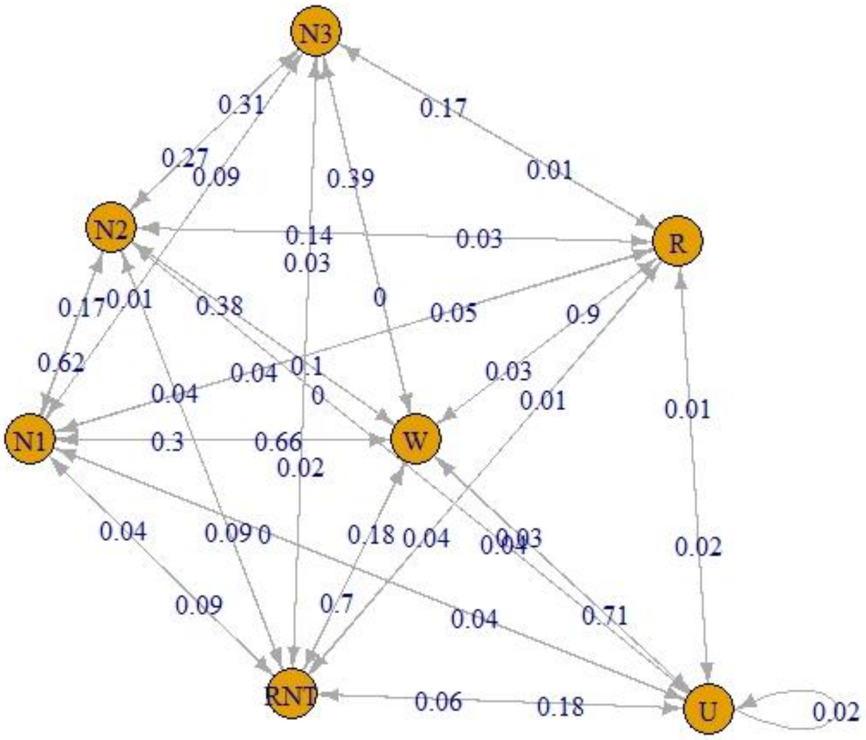

Figure 2 Bout transitions between sleep stages for all data

Table 5 *Overall number of bout transitions by sleep stage*

To

		N1	N2	N3	R	RNT	U	W
From	N1	0	471	5	30	27	0	227
	N2	107	0	177	88	22	1	249
	N3	17	60	0	33	6	0	74
	R	9	6	2	0	0	0	166
	RNT	20	20	4	10	0	5	160
	U	2	2	0	1	9	0	36
	W	607	88	3	23	165	18	0

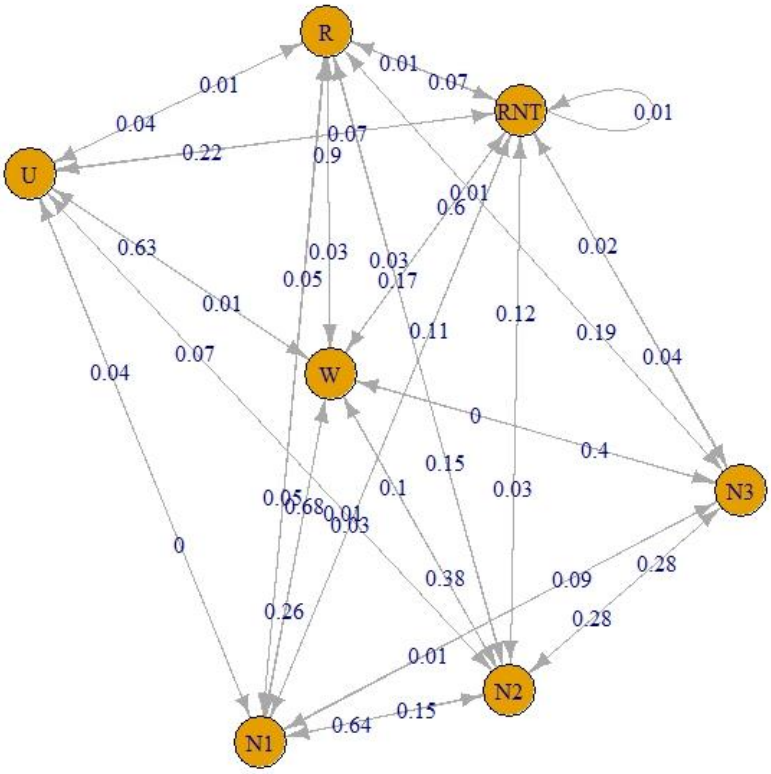

***Figure 3** Bout transitions between sleep stages only night-time*

***Table 6** Overall number of bout transitions by sleep stage during night-time recordings*

		To						
		N1	N2	N3	R	RNT	U	W
From	N1	0	330	5	28	17	0	137
	N2	69	0	126	66	14	1	174
	N3	12	39	0	27	5	0	55
	R	7	5	2	0	0	0	137
	RNT	17	18	3	10	0	3	88
	U	1	2	0	1	6	0	17
	W	413	59	3	21	103	7	0

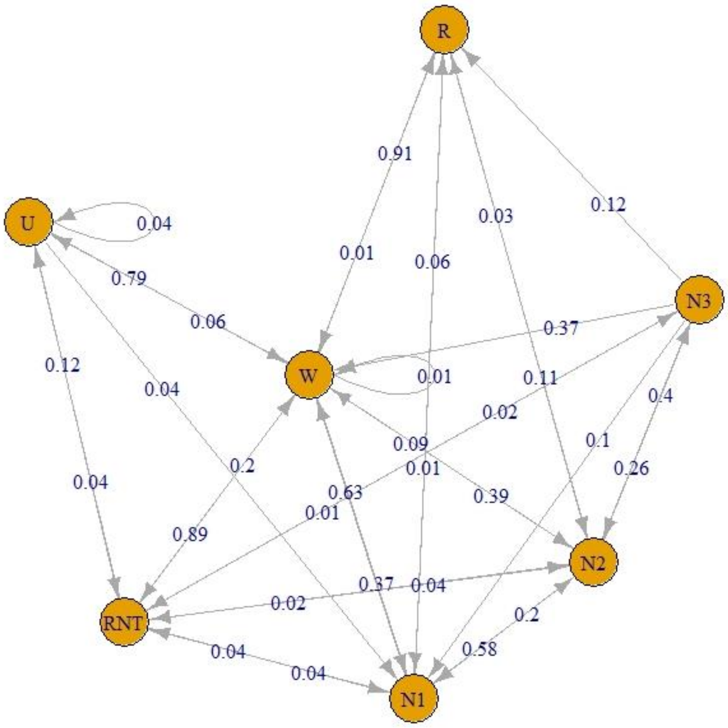

***Figure 4** bout transitions between sleep stages during the day*

***Table 7** Overall number of bout transitions by sleep stage during daytime recordings*

		To						
		N1	N2	N3	R	RNT	U	W
From	N1	0	141	0	2	10	0	90
	N2	38	0	51	22	8	0	75
	N3	5	21	0	6	1	0	19
	R	2	1	0	0	0	0	29
	RNT	3	2	1	0	0	1	72
	U	1	0	0	0	3	0	19
	W	194	29	0	2	59	8	0

Daily Sleep Stage Patterns

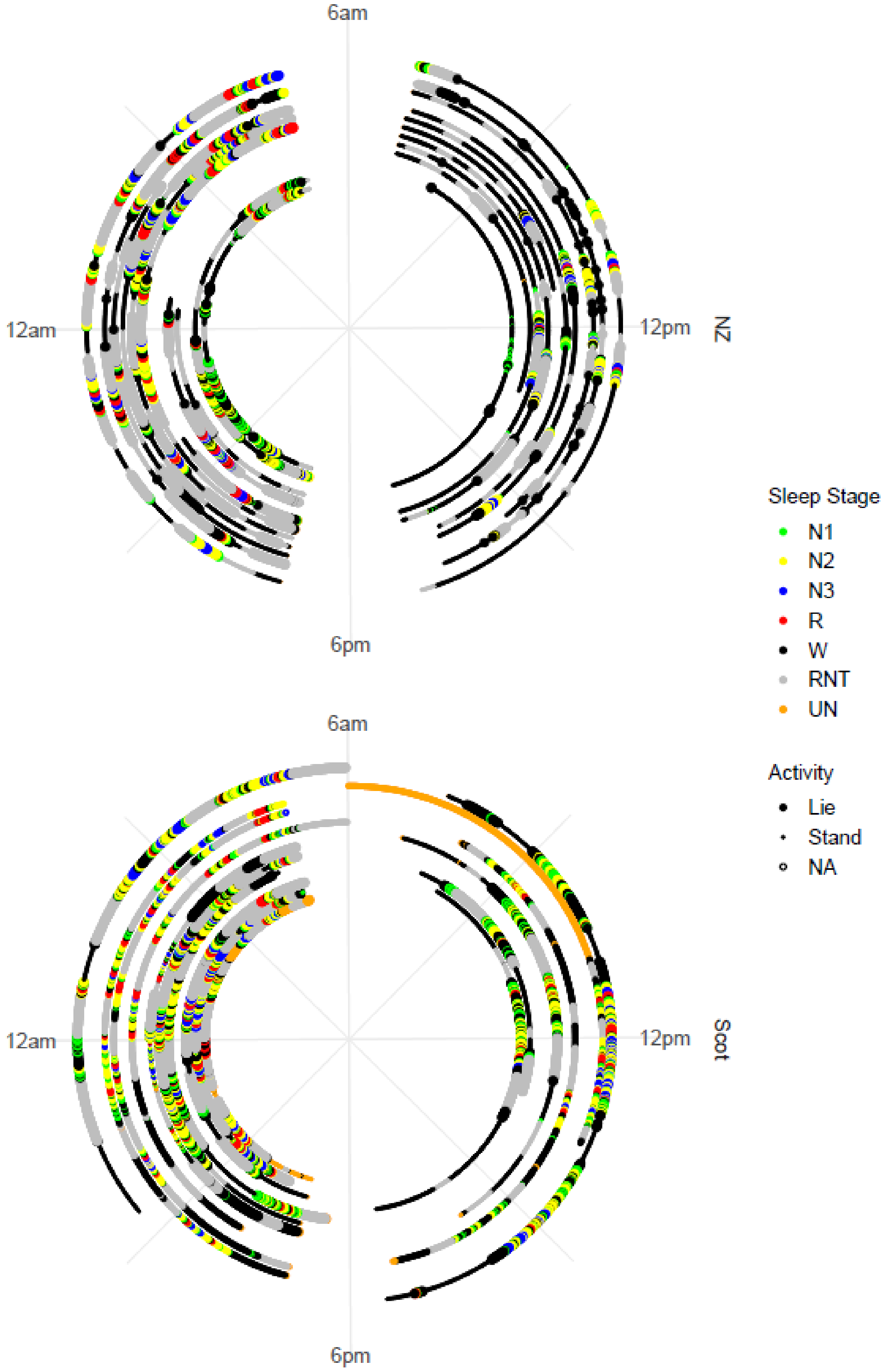

Figure 5 *Breakdown of each recording by sleep stage and standing or lying behaviour for NZ (top) and Scottish (bottom) cows.*

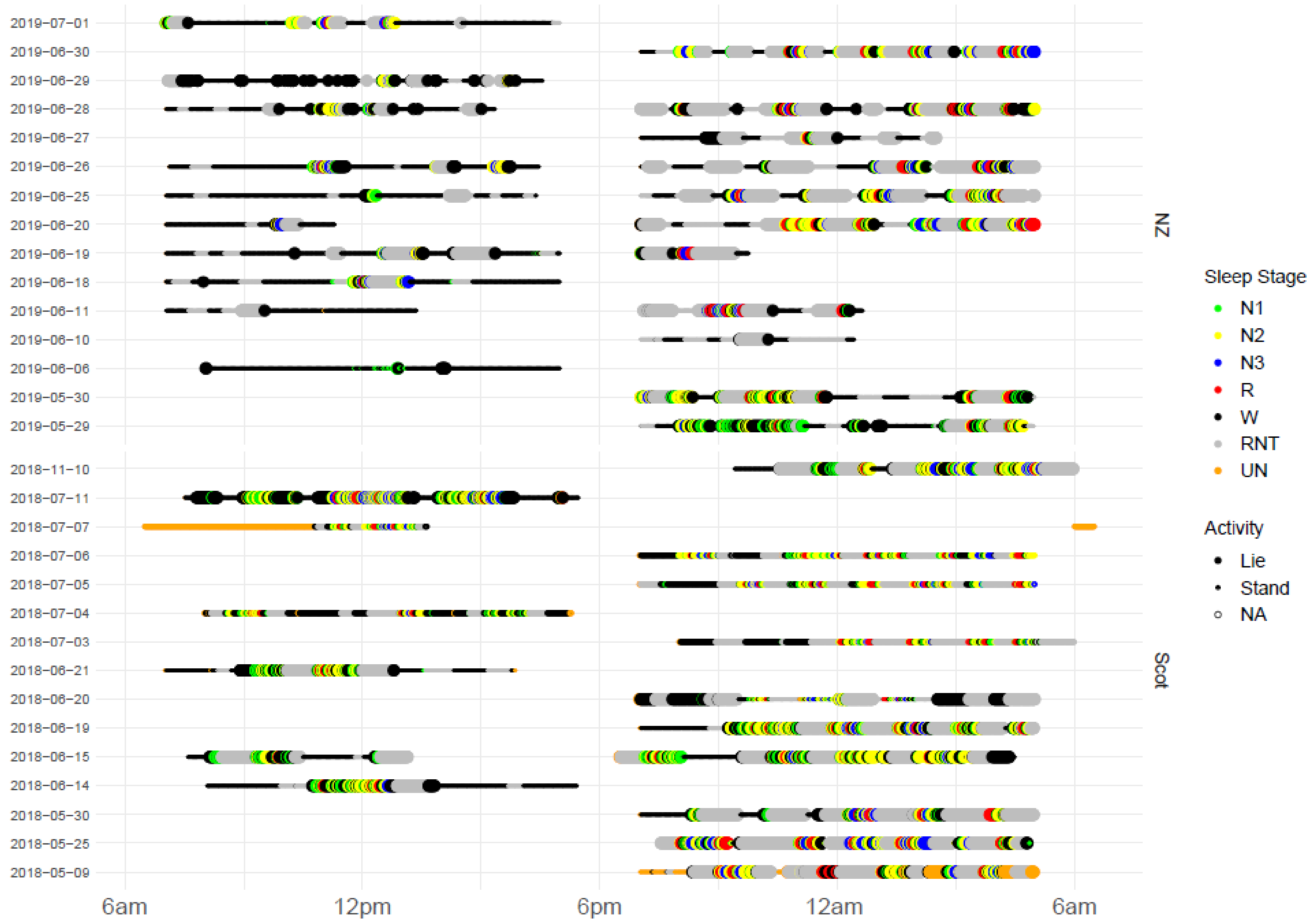

Figure 6 A diagram of each scored recording by sleep stage and activity

APPENDIX B

Conference Abstracts

CAN WE DETERMINE COW SLEEP STAGES FROM LYING POSTURE?

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Sleep is an essential function for all animals and its importance is increasingly being discussed in the human literature. Sleep restriction can have severe implications for many important health and welfare domains such as mental and physical health, immunity, development, and social interactions. Little is known about the importance of sleep for livestock welfare. This is mainly due to difficulties in accurately determining when an animal is asleep. The gold standard to stage sleep in animals is to use polysomnography (PSG); using electroencephalography (EEG) to record brain activity, electrooculography (EOG) for eye movements and electromyography (EMG) for muscle tone, however this method is delicate and impractical to apply in a large-scale study with unrestrained livestock.

Cows must lie down to sleep, therefore factors in the environment that affect cows lying behaviour are likely to have a significant impact on sleep. Cows adopt a characteristic tucked position with the neck turned and resting on the flank or ground and eyes closed, and occasionally will lie in a completely laterally recumbent posture. Previous studies in dairy calves have indicated that head position could be used with some accuracy to predict sleep stage in calves however it could not be used reliably in adult cows. The aim of this project was to continue to investigate the use of lying position and head posture in the prediction of cow sleep stages.

PSG recordings were made from 5 non-pregnant, non-lactating dairy cows managed individually in a 5m x 5m deep bedded straw pen at the SRUC Acre Head Research farm in Dumfries, Scotland. Readable data from 164hrs of recordings were scored into five stages of sleep from light to deep from PSG traces in 30 second epochs. Postures and head positions were scored from corresponding video recordings.

When in REM sleep tucked posture with head resting was observed in 78.51% of epochs, lateral lying in 14.8% of observations and other in 6.68%. However, REM sleep made up only 44.08% of all observations in the tucked posture while 47.91% of epochs were scored as light sleep or awake. REM sleep position varied between cows with some cows almost exclusively achieving this stage in a tucked position while another obtained almost half of scored REM sleep epochs in a laterally recumbent position. Non-REM deep sleep was observed in 58.84% of epochs when the cow was lying upright with the head low, however NREM sleep made up only 6.39% of all lying with head low epochs.

This data supports previous findings that lying posture and head position cannot be used accurately to estimate sleep stage in adult dairy cows. There may be individual preference for lying postures in deep sleep stages between cows, therefore estimates of sleep from postural data alone could be inaccurate between animals. This research also reinforces the need for further research into practical alternative methods of assessing sleep stages in large animals that can be applied in a larger scale.

USING MACHINE LEARNING TO PREDICT SLEEP STAGES FROM MUSCLE ACTIVITY AND HEART RATE IN DAIRY COWS

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Little is known about the importance of sleep for cow welfare, mainly due to difficulties in accurately determining when an animal is asleep. The gold standard method - polysomnography (PSG)- involves recording electrophysiological traces of the brain (EEG), eyes (EOG) and muscles (EMG), but is fragile and impractical to apply in large-scale studies or pastoral environments. Algorithms and devices such as smart watches have been developed for humans to predict sleep stages using movement and physiological changes. The aim of this project was to determine if similarly, heart rate (HR) and EMG activity alone could predict sleep stages accurately compared to PSG in dairy cows. Assessing the use of the new measures in both indoor-housed and pasture environments would allow the methodology to be used in either environment.

Ethical approval was obtained prior to the start of the study. Six pregnant dry cows (New Zealand) and six non-pregnant dry cows (Scotland, UK) were fitted with PSG-recording equipment and a Polar HR monitoring belt. During recordings the cows were managed individually in a 5m x 5m indoor straw (UK) or 10m x 10m outdoor pasture enclosure (NZ), but always maintained audio-visual contact with group mates. Four sleep stages (Rapid Eye Movement (REM) and Non-REM (N1, N2, N3)), wakefulness and rumination were scored in 30 second epochs from the PSG traces and corresponding statistics were generated from the filtered HR and EMG traces. 192.7 hours of good quality data were used to build and test a machine learning model using the Orange machine learning platform. The performance of two machine learning algorithms (Neural Networks (NN) and Random Forest (RF)) was compared using ten-fold cross validation. NN performed best and was able to predict overall sleep stages with an area under the receiver operating curve (AUC) of 92.5% and classification accuracy (CA) of 82.3%. In predicting individual sleep stages, the model had more success identifying REM sleep (AUC 92.4%, CA 95.8%) and wakefulness (AUC 94.7%, CA 88.4%) than Non-REM stages such as N3 (AUC 90.8%, CA 85.25).

Using HR and EMG data alone, the model was able to accurately predict sleep stages in the range of similar human models. These results are promising and the identification of sleep using non-PSG methods will allow us to investigate sleep in more commercially relevant settings.

APPENDIX C

Papers Formatted for Publication

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Heart Rate and Heart Rate Variability Change with Sleep Stage in Dairy Cows

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Simple Summary: The amount of sleep acquired and changes to patterns of sleep could be a useful tool to assess cow welfare, particularly in response to changes or stressors in their environment. However, the current most accurate method to assess sleep, polysomnography (PSG), is difficult and time consuming. In humans, heart rate (HR) and variability in time between heart beats (HRV) can be used to identify sleep stages, and this could be a useful alternative to investigate sleep in cows. We compared measures of HR and HRV with PSG in two groups of dairy cows in different environments and investigated the effects of lying posture on these measures. We found that HR decreased with deepening sleep stages in both groups of cows, that rapid eye movement sleep (REM) was associated with higher HRV and that HR and HRV also changed with different lying postures. The patterns of differences between sleep stages were similar between the two groups of cows. Our results suggest that HR and HRV change with sleep stages in cows and that these measures could be a useful, and more easily applied, method of assessing sleep stages in dairy cows.

Abstract: Changes to the amount and patterns of sleep stages could be a useful tool to assess the effects of stress or changes to the environment in animal welfare research. However, the gold standard method, polysomnography PSG, is difficult to use with large animals such as dairy cows. Heart rate (HR) and heart rate variability (HRV) can be used to predict sleep stages in humans and could be useful as an easier method to identify sleep stages in cows. We compared the mean HR and HRV and lying posture of dairy cows at pasture and when housed, with sleep stages identified through PSG. HR and HRV were higher when cows were moving their heads or when lying flat on their side. Overall, mean HR decreased with depth of sleep. There was more variability in time between successive heart beats during REM sleep, and more variability in time between heart beats when cows were awake and in REM sleep. These shifts in HR measures between sleep stages followed similar patterns despite differences in mean HR between the groups. Our results show that HR and HRV measures could be a promising alternative method to PSG for assessing sleep in dairy cows.

Keywords: dairy cows; heart rate; sleep; heart rate variability; polysomnography

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1. Introduction

Two main stages of sleep exhibited by animals are known as rapid eye movement sleep (REM) and non-REM sleep. Non-REM sleep has been associated with restorative functions in the body and brain, for example, the clearance of potentially harmful toxins produced by normal cellular function [1]. REM sleep has been associated with memory, learning and dreaming [2]. Changes to the amount and patterns of sleep stages could be used to assess animal welfare, as these aspects of sleep are known to be affected by factors such as environmental conditions, stressful occurrences during the day, pain or illness [3]. For example, after moving into an unfamiliar environment, cows were found to spend less time lying in postures associated with sleep than their baseline, which could be an indication of stress [4]. However, in dairy cows, without using neuro-electrophysiological methods, it is difficult to accurately identify sleep from wakefulness, let alone different sleep stages.

As sleep is a homeostatic function originating in the brain, the most accurate way to study it is through polysomnography (PSG), the study of multiple electrophysiological signals, namely brain activity, eye movements and muscle activity [5]. PSG can be successfully used to study sleep in calves [6] and in adult cows [7,8]; however, it is costly, the equipment is fragile, and interpretation of the signals is time-consuming [7]. Being able to identify sleep in dairy cows with other more easily applied or less invasive devices would be beneficial not only for the cow's comfort and welfare, but also for ease of application by researchers, thus facilitating the study of the sleep of cows and opening several new avenues for investigation of the effects of sleep loss or importance of sleep for cows.

During sleep and different sleep stages, changes occur in the regulation of the mammalian autonomic nervous system (ANS) and its subdivisions, the parasympathetic (PNS) and sympathetic nervous systems (SNS), affecting many functions such as heart rate, respiration rate and body temperature [9]. Specifically, during REM sleep, there is variability in ANS activity, leading to more variability in the associated physiological functions, whereas in non-REM sleep stages, there is more activity of the PNS while SNS activity is reduced [9]. Because the ANS affects the heart, measures of heart rate (HR) and heart rate variability (HRV: the measurement of the variability in the time between successive heart beats) can be used as a way to identify activation of the PNS and SNS [10]. In humans, changes in HR and HRV have been used to accurately identify and differentiate between sleep stages [11,12]. HRV can be quantified with different methods. Time domain indices of HRV identify differences in the time between successive heart beats or inter-beat-interval (IBI) while frequency domain indices classify the signal into frequency bands [13].

The study of HRV in cow welfare to date has focussed mainly on the application of HR and HRV to identify and assess stress. HR and HRV were found to be affected by severe lameness which may cause chronic stress in cattle [14]. Calves being disbudded without local anaesthetic showed an increase in frequency domain metrics of HRV [15]. HR and HRV has also been used to identify positive interactions in dairy cows, and social licking between cows was found to reduce heart rate in receivers [16]. Body posture has been found to affect HR and HRV measures. Heart rate was lower and variability in time between heart beats was higher in cows lying down compared to when standing [17]. However, to our knowledge, investigations of HR and HRV during sleep in cows have not been done. In previous work, we have found that sleep occurs when cows are lying down, but specific lying postures could not be used reliably to identify sleep stages compared to PSG in dairy cows. Furthermore, housing conditions have been shown to affect the relationship between lying postures and sleep [18].

It is possible that HR and HRV could be used to identify sleep stages in cows. The equipment required to assess HR and HRV is less invasive and more easily applied than equipment used for PSG. Therefore, the objective of this study was to determine if HR and HRV differ between sleep stages in dairy cows, and to determine if this is repeatable between cows in different areas, housing conditions and lying postures.

2. Materials and Methods

2.1. Ethical Statement

The study was designed in accordance with the relevant guidelines and legislation in both Scotland and New Zealand (NZ) where the studies took place. Ethical approval was obtained from the UK Home Office (Project Licence P204B097E), SRUC Animal Ethics Committee (Ref. ED AE 03-2018) and Ruakura Animal Ethics committee (AE 14708) prior to the start of animal manipulations.

2.2. Cows and Housing

Twelve cows were recruited for this study from two locations. Six non-lactating and non-pregnant Holstein cows (age 3.9 ± 0.7) from the Acrehead unit of SRUC's Dairy Research Centre (Dumfries, Scotland) and six, three-year-old, non-lactating, pregnant Kiwicross (Friesian × Jersey) cows from the DairyNZ Lye Farm (Newstead, NZ) were used. The small sample size was necessary due to the time-intensive methods required to habituate the cows to the recording devices. Non-lactating cows were selected to avoid disruptions to the cow's sleep patterns due to fetching for milking and the risk of damage to recording devices in the milking parlour. The Scottish cows, destined to be culled from the herd due to reduced fertility, were healthy during the trial.

The cows were managed in a large group pen and moved into a smaller adjacent 'test' pen individually for recording sessions (Figure 1). The Scottish cows were held on deep-bedded straw in a barn. The group pen measured 20 m × 5.2 m and test pen 5.2 m × 5.1 m. The cows were fed silage and always had access to water. The NZ cows were managed outdoors in a large paddock. They were able to graze and were provided with silage ad libitum and always had access to water. The group pen measured 44 m × 29 m and was created with live electric fencing. The 10 m × 10 m test pen was created with non-live electric fencing tape, to prevent potential interference with the electrophysiological recordings. The fencing set-up for both group and test pens could be moved around the paddock when ground conditions became wet or muddy. In both locations, a 2 m buffer zone was created between the test pen and group pen, to inhibit contact and reduce damage to recording devices from social interactions.

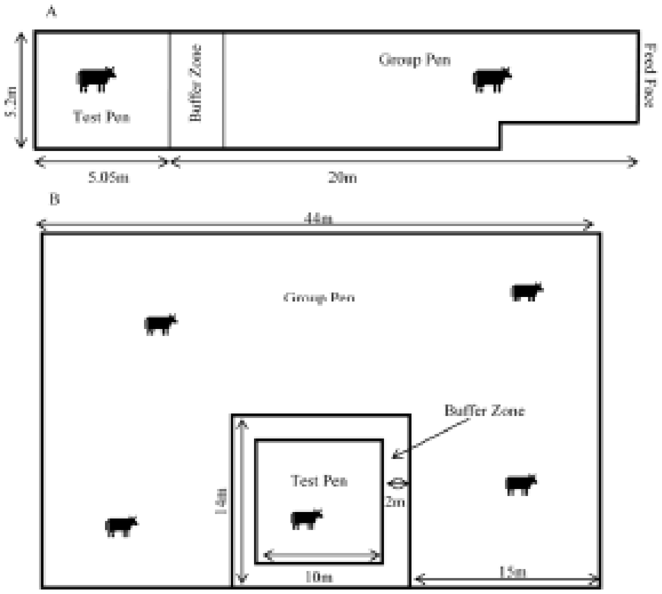

Figure 1. Diagrams of group and test pen design in the Scottish indoor-housed study (**A**) and in the NZ outdoor pasture study (**B**). During recordings, the test cow was moved into the test pen. When not recording, the cow was moved back into to the group pen.

The cows were fitted with the recording devices and moved into the test pens individually for a maximum of 7 days. The devices and recording gear were downloaded, re-charged and re-set twice daily. Devices were removed if the cow showed signs of skin or behavioural irritation or in the case of forecasted heavy rain (NZ outdoor group).

2.3. *Heart Rate Recording*

HR and HRV were measured using a polar RS800 CX watch and Polar equine monitoring belt (Polar Electro Oy, Kempele, Finland). Patches of hair at the electrode locations were clipped and the electrodes were generously coated with ultrasound gel (Aquasonic 100 gel, Parker Laboratories, Fairfield, NJ, USA) to improve contact with the skin and signal transmission. The belt and watch were checked frequently and adjusted as needed throughout the recording. An elastic surcingle was attached over the belt to keep it tight to the skin. The clasps of the Polar belt and surcingle were padded with felt and wrapped in cohesive bandage to reduce the chance of irritation to the cows and also reduce the chance of the surcingle loosening throughout the recording. The watch was synchronized to the recording computer's time and was programmed to record heart rate and R-R intervals which are used for HRV calculations. R-R intervals are the time (in milliseconds) from the R peak of one heartbeat to the R peak of the next heartbeat.

The data were downloaded and analysed using Polar Pro Trainer (v5.35.160) and artefacts in the R-R data were filtered and corrected using moderate filter power. Only traces containing less than 1% of identified errors were used in the analysis. Filtered data were exported and HR and HRV statistics were calculated in 30 s intervals (epochs) corresponding to the scored PSG data. Only time domain features of the HRV were calculated since frequency domain features of the HRV may not be an accurate representation of the data in such small time periods [19]. Time domain features included mean HR (in beats per minute—BPM), root mean square of successive differences of the R-R signal (RMSSD), and standard deviation of the R-R signal (SDRR) in 30 s epochs.

2.4. *PSG Recording and Sleep Scoring*

2.4.1. PSG Recording Protocols

PSG were recorded as described by Hunter et al. (2021) [20]). Pre-gelled adhesive snap ECG electrodes (Natus Neurology, Ottawa, ON, Canada) were used to record four EEG, a reference (REF), patient grounding (PGND), and two EOG and two EMG channels from the cows. Lead wires were snapped on, bundled down the neck and plugged into the Embletta MPR PG + St proxy PSG recording device (Embla Systems, Ottawa, ON, Canada). The device was placed in a padded plastic box within a pouch sewn to the elastic surcingle covering the HR monitor belt. The device was programmed, data were downloaded, and traces processed and scored using RemLogic 3.4.3 software (Embla Systems, Ottawa, ON, Canada). Good quality recordings, which had a minimum of one complete EEG, EOG and EMG trace each with good impedance (1–14kHz), minimal artefacts and with good quality corresponding HR traces were used in the analysis. Recordings lasted a maximum of 10 h due to device memory limitations.

2.4.2. Sleep Scoring

The good quality traces were scored according to criteria developed from a combination of previous cow sleep EEG studies [6,7,21] as well as human sleep scoring criteria [22]. Five stages of sleep and wakefulness were scored: Awake (W), REM (R) and Non-REM (which was subdivided into 3 stages, light N1 and N2, and deep N3). Rumination was also scored from the PSG as substantial artefacts due to jaw muscle movements when chewing obscured the actual signals of the traces and it was impossible to tell what stage the cow was in during that time. Intra-observer accuracy was calculated using "irr" package [23] in R (v4.0.5) using Cohen's Kappa with $\kappa = 0.83$ and overall agreement of 89.4% indicating good agreement [24].

2.4.3. Lying Postures

Lying postures were identified from video recordings made from four surveillance cameras equipped with automatic infra-red night vision capability (Geovision monitoring system, Viewlog, GeoVision Inc., Taiwan (Scottish Cows)) and Vivotek ND9541P H.265 NVR (Vivotek Inc., Taiwan) (NZ Cows)). Lying postures, with head held up above the point of the shoulder (UP), head held below the shoulder (HL), head resting on the ground to the front (HF), neck turned with head resting on flank or "tucked" (T), lateral lying or "flat out" (FO) and moving (MV) as well as not scored (NS) (Table 1), were scored instantaneously every 30 s corresponding to the start of the PSG and HR epochs. Intra-observer reliability was conducted in R (v4.0.5) [25] using the Cohen's kappa in the 'irr' package [23] and the kappa statistic was κ = 0.95 demonstrating a high level of agreement [24].

Table 1. Behavioural ethogram for scoring lying postures in dairy cows, including head positions and photographs from surveillance videos.

	Head Up (UP) Lying sternally recumbent with head held up
	Head Low (HL) Lying sternally recumbent with head held low
	Head Resting Front (HF) Lying sternally recumbent with the head and or neck resting on the ground
	Tucked (T) Lying sternally recumbent with head turned and resting on the flank
	Flat-out (FO) Lying laterally with legs extended and head and neck resting on the ground

2.5. Data Analysis

Scored sleep stages, lying postures and heart rate data were aligned by time stamps. In cows, sleep occurs when lying down [21], therefore only epochs identified as lying were included in the analysis. Epochs with posture 'not scored' (NS) due to observer inability to accurately observe behaviour or other extraneous circumstances were also removed from the dataset. As the stages of sleep or wakefulness could not be determined while ruminating, these epochs were also removed.

We fitted a mixed-effects model to determine if the cow's HR changed by sleep stage using the 'lme4' [26] and 'lmertest' [27] packages in R (v4.0.5) [25]. The fixed effects were study (country), sleep stage and their interaction. We included recording day nested within individual cow ID as random factors. We then used the same model with each of the remaining variables, and RMSSD and SDRR as the response variables. Using the 'predictmeans' [28] package we calculated the predicted means, standard error of the means (SEM) and least significant differences (LSD).

We then re-ran the same models, now including the cow's lying posture as a fixed effect with interaction with study and calculated predicted means of cow's HR and HRV measures by lying posture and study.

3. Results

Overall, with rumination, standing, and unscored lying behaviour removed, 1968 epochs totaling 16.4 h of good quality data were obtained from 10 cows in 29 recordings days. Data from one Scottish and one NZ cow were removed as they each had only one limited good quality recording that did not contain any lying periods. The data set was skewed towards more time in the awake (W) state, as 629 epochs were scored as W, 315 epochs in N1, 593 epochs in N2, 197 epochs in N3 and finally 234 epochs in REM (Table 2).

Table 2. Count of the total number of epochs of data in each posture by sleep stage (tucked (T), head resting front (HF), head low (HL), head up (UP), moving (MV) and flat out (FO)) and study country.

	NZ							Scot						
	T	**HF**	**HL**	**UP**	**MV**	**FO**	**Total**	**T**	**HF**	**HL**	**UP**	**MV**	**FO**	**Total**
W	24	2	67	116	14	1	224	42	4	133	157	49	20	405
N1	4	2	34	58	3	0	101	20	1	103	52	13	25	214
N2	9	4	61	120	0	0	194	51	13	217	46	6	66	399
N3	8	2	21	47	0	0	78	17	1	72	15	3	11	119
R	90	0	0	1	0	0	91	114	1	4	3	1	20	143
Total by Posture	135	10	183	342	17	1	688	244	20	529	273	72	142	1280

3.1. *Lying Posture*

The HR and HRV parameters changed with specific body posture while lying. In the Scottish cows, moving and lying flat out postures resulted in significantly higher mean heart rate (MV = 56.43 ± 3.17 bpm, FO = 55.53 ± 3.15 bpm) than all other postures (lying with the head up, or low, or resting on the ground or with the head tucked) (Figure 2). Flat out lying was rare in the NZ data, with only one epoch over all observations. Moving was also associated with a higher mean HR in the NZ group (84.22 ± 3.41 bpm). In the NZ cows, tucked posture was also associated with significantly higher RMSSD values than the head low, head up and moving postures, indicating more variability in the time between successive heart beats. Similar results were found with RMSSD in the Scottish group, who had higher RMSSD in T compared to HL ($p = 0.007$, df = 1936, $t = 2.7$), UP ($p = 0.0017$, df = 1936, $t = 3.15$) and lower compared to FO ($p = 0.0035$, df = 1936, $t = -2.92$). We also found a significant effect of sleep stage and its interaction with study location on the HR and HRV parameters. Table 2 shows the means for the different sleep stages.

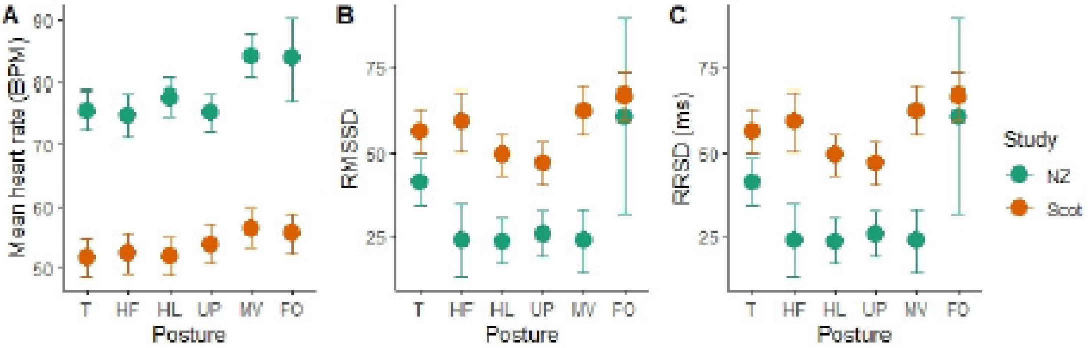

Figure 2. Plots of predicted means with error bars indicating standard error of the means for the mean heart rate in beats per minute (bpm) (**A**), the RMSSD (**B**) and SDRR (**C**) by lying postures (Tucked (T), head resting front (HF), head low (HL), head up (UP), moving (MV) and flat out (FO)) for the New Zealand group (NZ), Scottish group (Scot). Figure produced in R v4.0.5 using ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/index.html accessed on 3 May 2021).

3.2. *Mean HR*

After accounting for variation between cows and study days, we found a large effect of study group on mean HR. Mean HR was around 20 BPM lower in the Scottish cows than the NZ cows. After accounting for this variation, significant differences between sleep stages were evident. In both the indoor-housed Scottish group and outdoor-managed NZ cows, mean heart rate was significantly slower in the REM sleep stage compared to awake (W) (NZ: $p < 0.001$, df = 1934, t = 5.51) (Scottish: $p < 0.001$, df = 1934, t = 12.16). In the Scottish group, N2 and N3 stages were not significantly different from one another ($p = 0.09$, df = 1934, t = 1.68), but all others (W, N1, R) were. In the NZ group, W and N1 were not different from each other ($p = 0.46$, df = 1934, t = −0.74), and neither were N3 and REM ($p = 0.89$, df = 1934, t = 0.14). Overall, heart rate declined successively from W to N1 and then to N2, while N3 and REM were significantly lower than the other sleep stages.

3.3. *RMSSD-Variability between Successive Heart Beats*

As heart rates were significantly different between the study groups, it is unsurprising that they also had a significant effect of study on the RMSSD (Figure 3). As the mean heart rate was lower in the Scottish group, their RMSSD was 15–30ms higher than the NZ group, indicating longer inter-beat intervals (Table 3). Accounting for the random effects, we found significantly higher RMSSD values during REM sleep epochs, indicating more variability in the time between successive heart beats in this stage. In the NZ group, the RMSSD during REM sleep was significantly higher than W ($p = 0.0061$, df = 1937, t = −2.74), N1 ($p = 0.01$, df = 1937, t = −2.57) and N2 ($p = 0.0056$, df = 1937, t = −2.78) but not significantly different from N3 ($p = 0.18$). In the Scottish group, RMSSD during REM sleep was highly significantly different from all other sleep stages, which did not differ greatly from one another. However, N2 did differ significantly from W ($p = 0.002$, df = 1937, t = −3.09) and N1 ($p = 0.028$, df = 1937, t = −1.554). Overall, the time between successive heart beats during REM sleep was significantly more variable than the other stages. N3 was more variable than W but not compared to the other NREM stages (N1 & N2).

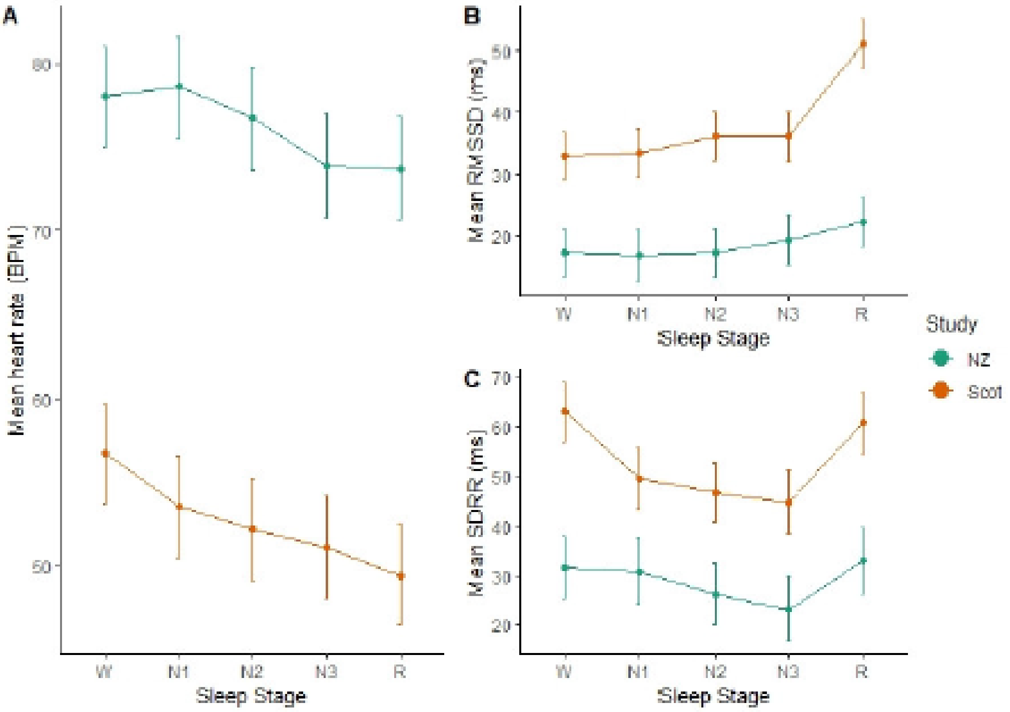

Figure 3. Plots of predicted means with error bars indicating standard error of the mean in each sleep stage for the NZ and Scotland groups for mean HR (**A**), RMSSD (**B**) and SDRR (**C**)). Figure produced in R v4.0.5 using ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/index.html. accessed on 3 May 2021)

Table 3. Table of predicted means ± standard error of the HR mean, RMSSD and SDRR for each of the stages, awake (W), Non-REM: N1–N3 and REM sleep overall data and by study group in NZ and Scotland (SC).

Sleep Stage	W	N1	N2	N3	REM
HR Mean					
Predicted Mean NZ	78.03 ^{A} ± 3.05	78.57 ^{A} ± 3.09	76.72 ^{B} ± 3.06	73.90 ^{C} ± 3.10	73.78 ^{C} ± 3.09
Predicted Mean SC	56.77 ^{D} ± 3.04	53.56 ^{E} ± 3.05	52.20 ^{F} ± 3.04	51.14 ^{F} ± 3.08	49.48 ^{G} ± 3.07
RMSSD					
Predicted Mean NZ	17.15^{A} ± 3.97	16.80 ^{A} ± 4.12	17.14 ^{A} ± 3.99	19.22 ^{AB} ± 4.18	22.14 ^{BC} ± 4.14
Predicted Mean SC	32.92 ^{CD} ± 3.82	33.41 ^{DE} ± 3.88	36.03 ^{F} ± 3.83	35.96 ^{EF} ± 3.98	50.96 ^{G} ± 3.94
SDRR					
Predicted Mean NZ	31.74 ^{AB} ± 6.22	30.95 ^{ABC} ± 6.60	26.30 ^{AC} ± 6.27	23.32 ^{C} ± 6.75	33.08 ^{ABD} ± 6.63
Predicted Mean SC	62.93^{E} ± 6.03	49.61 ^{D} ± 6.19	46.69 ^{BD} ± 6.04	44.83 ^{BD} ± 6.42	60.71 ^{E} ± 6.34

$^{A–G}$ Means within each HR/HRV measure without a common superscripted letter are significantly different at $p < 0.05$.

3.4. *SDRR-Total Variability of Time between Heart Beats*

There were differences between the groups, but this was not as wide as for the other variables. SDRR was higher for the awake and REM stages compared to the other stages, indicating that there was higher variability in the overall time between heart beats for these stages. In the NZ cows, SDRR during REM sleep was significantly higher than N3 ($p = 0.0288$, df = 1947, t = −2.1882), but not the other stages. N3 was significantly lower than W ($p = 0.0337$, df = 1947, t = 2.1254), however N3 was not significantly different from the other stages. In the Scottish group, SDRR was not significantly different between W and REM ($p = 0.4422$, df = 1947, t = 0.7687), but these stages were significantly higher than N1, N2 and N3 that were not significantly different from one another.

4. Discussion

Our results show that cardiac outputs could be useful in assessing sleep stages in dairy cows. However, we found major differences in mean HR between the two groups of cows, which may be due to different cow characteristics. Understandably, despite replication in data collection methods, there were marked differences in the housing, breed, size, physiological stage, and diet of the cows in each study location. The NZ cows were all in late pregnancy, whereas the Scottish cows were non-pregnant and non-lactating. Late pregnant heifers and cows have been found to have higher mean HR than earlier on in pregnancy [29]. While there was not a particularly large difference in cow age, there was a difference between cow size and breed. The Scottish cows were very large Holstein cows, and the NZ cows were much smaller being Jersey-Holstein crosses (Kiwi-cross). Other studies have found significant differences in HR and HRV measures between different breeds (Brown Swiss and Simmental) when standing, lying and milking [30]. Body size is also known to affect HR and HRV, and a decrease in HR was found with increasing weight in horses and ponies [31]. The Scottish recordings were made indoors in spring/summer months with a daytime temperature average of 15.2 °C (range 8–22 °C) and overnight temperature of 11.2 °C (range 5–22 °C). The NZ recordings were made outdoors over winter with average an average daytime temperature of 10.2 °C (range 2–18 °C) and overnight average of 8.4 °C (range 2–14 °C). Seasonal thermal stress has been found to affect behaviour, stress and immune response in dairy cows [32,33], and increasing temperature humidity index has been associated with decreased HRV measures in sheep and goats [34]. Although the cows in the winter conditions in NZ had higher HR and lower HRV than cows in summer conditions in Scotland, the environmental conditions could have affected the HR and HRV activity in this study.

Importantly, despite these group differences, we found that HR and HRV changes with sleep stages in both groups and clearly, Figure 2 shows that the differences are in the same direction. These results indicate that the patterns of the changes in HR and HRV measures between the sleep stages are stable and as such these measures could be used with all cows, although further research is needed to assess if these patterns are also observed in lactating cows and cows in other stages of pregnancy.

Surprisingly, we found that mean HR during REM sleep was lower than when awake and in the lighter NREM sleep stages (N1 and N2). This is different than results in humans, where HR has been found to decrease with the progression of NREM sleep stages, and speeds up again in REM sleep [35]. However, similar results with an overall lower heart rate during REM sleep were also found in dogs [36]. Despite the lower mean HR, HRV measures of RMSSD and SDRR were higher in REM sleep, indicating more variation between heart beats. This observation is similar to that shown for HRV patterns during human sleep, where HRV tends to be more variable when awake and in REM sleep than during N3 and other NREM sleep stages [37]. Mean HR and RMSSD may be useful to distinguish between awake (W) and REM stages; however, they are not particularly useful to distinguish between NREM stages (N1, N2, N3). SSDRR was useful to identify N3 stages in both groups as it was significantly lower. These patterns of differences in sleep stage could be useful in future applications to predict sleep stage of dairy cows, particularly if prior to recording, a lying awake baseline could be specified. Then sleep stages could be identified or predicted from differences from that baseline.

A previous study has found that body position was associated with difference in HR and HRV measures in cows [17]; however, they did not specify body posture while lying and were unable to identify awareness levels. We found that the specific posture that cows adopted during lying affected their HR and HRV, and in particular that epochs identified as being in the flat out (lateral lying) posture and epochs with the head moving resulted in higher HR and more variability in the HRV. As moving is a physically active behaviour, this activity may have had a carry-over effect on the heart rate for an extended period. Therefore, an epoch in which the cow moved her head at the start may have higher HR across that epoch and into the next. Flat out posture was rarely observed in the NZ group, and only scored once, and even then, was only observed as a transition behaviour between other postures. In the Scottish group, flat out postures were far more commonly observed, and most often occurred while the cow was in N2 sleep as well as awake and in N1. It is unclear if the increased HR in this posture was due to the position of the body which could have facilitated a faster movement of blood, or because most epochs scored as flat out happened to occur in sleep stages that had higher heart rates. In the NZ group almost all REM sleep occurred in the tucked posture. The tucked posture was found to have significantly higher RMSSD; however, since REM sleep was also found to have higher RMSSD it is likely that the effect of the posture on the HRV measure was more likely due to the sleep stage in this case.

The intra-observer reliability for sleep scoring was 89%, which according to interscorer reliability in human sleep studies is very good [38,39]. However, there is still some possibility that the 11% uncertainty in sleep scoring was a contributing factor to the variability of the HR and HRV measures within sleep stages. Additionally, we analysed the HR data in 30 s epochs, specifically to correspond to sleep scoring. The 30 s epoch is a standard practice in scoring sleep stages from PSG, as it corresponds well to the structure of human sleep, containing fewer stage shifts than longer epochs which would be more likely to contain many stage shifts[40]. Despite shorter epoch length, some mid-epoch stage shifts could still have occurred. In these instances, although the PSG was scored one way, the HR measures could have reflected another stage, and this could also be another source of variability in the HR and HRV measures within sleep stage.

Similarly, the HR and HRV data may have also been influenced by the short epoch windows. Typically, human HRV measures are conducted in 5 min increments, although ultra-short windows such as 30 s windows have been found to be acceptable for the assessment of HRV at rest in humans [41]. Bouts of cow sleep stages can typically be quite short [7,21] and thus multiple stage shifts would be captured in a longer epoch length of 5 min. This was a major reason for choosing to analyse the HR and HRV in ultra-short windows. However, some have questioned the accuracy of windows shorter than 2-min for the analysis of HRV in human athletes [19]. RMSSD measurements in even shorter 10 s windows were also found to correspond well to standard longer intervals in humans, but SDRR did not [42]. Therefore, the short time window selection could have affected the accuracy of the cow HRV RMSSD and SDRR measurements. HR and HRV may be useful for the assessment of sleep stages in dairy cows, however, further investigation into the validity of ultra-short HRV measures in dairy cows and additional validation with PSG is needed.

5. Conclusions

We have shown that sleep stage is associated with changes in HR and HRV in dairy cows. Mean HR was significantly lower in the indoor-housed, non-pregnant, and nonlactating cows compared to pregnant, dry, outdoor managed cows. We also found that mean HR decreased with sleep depth, SDRR was more variable in awake and REM states, and RMSSD was significantly higher in REM sleep than the other stages. These results indicate that HR and HRV could be a useful measure for the future identification of sleep stages in dairy cows using less invasive devices than PSG, making sleep research for animal welfare more accessible.

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Institutional Review Board Statement: The study was designed in accordance with the relevant guidelines and legislation in both Scotland and New Zealand where the studies took place. Ethical approval was obtained from the UK Home Office (Project Licence P204B097E), SRUC Animal Ethics Committee (Ref. ED AE 03-2018) and Ruakura Animal Ethics committee (AE 14708) prior to the start of animal manipulations.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, L.B.H., upon reasonable request.

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Machine learning prediction of sleep stages in dairy cows from heart rate and muscle activity measures

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Sleep is important for cow health and shows promise as a tool for assessing welfare, but methods to accurately distinguish between important sleep stages are difficult and impractical to use with cattle in typical farm environments. The objective of this study was to determine if data from more easily applied non-invasive devices assessing neck muscle activity and heart rate (HR) alone could be used to differentiate between sleep stages. We developed, trained, and compared two machine learning models using neural networks and random forest algorithms to predict sleep stages from 15 variables (features) of the muscle activity and HR data collected from 12 cows in two environments. Using k-fold cross validation we compared the success of the models to the gold standard, Polysomnography (PSG). Overall, both models learned from the data and were able to accurately predict sleep stages from HR and muscle activity alone with classification accuracy in the range of similar human models. Further research is required to validate the models with a larger sample size, but the proposed methodology appears to give an accurate representation of sleep stages in cattle and could consequentially enable future sleep research into conditions affecting cow sleep and welfare.

Animals are driven to sleep and it is vital that enough restful sleep is achieved to feel replenished[1]. Feelings of exhaustion, tiredness and sleeplessness can impact negatively on animal welfare[2]. Health can also be significantly impacted by sleep loss (sleep deprivation or restriction), which can result in activation of the immune and inflammatory systems[3] and also influence pain sensitivity and perception[4] in both humans and animals.

We know very little about the importance of sleep and the effects of limited or poor-quality sleep for dairy cows. Broadly, it is likely that factors affecting lying behaviour will also influence

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sleep, as cows must lie down to achieve it[5]. Sleep can be affected by stressful experiences during the day[6]. Therefore, changes to sleep patterns or total sleep time in cattle could be useful indicators for stress and other welfare concerns. The ability to identify sleep stages accurately could enable research on the effects of sleep loss for cows and could be useful to inform management practices such as determining rest intervals during long-haul transport or management of cattle during wet weather (i.e. on standoff pads).

Sleep consists of two main types: rapid eye movement (REM) and non-REM (NREM) sleep. The most accurate method of identifying sleep types is polysomnography (PSG)[7,8], which consists of a combination of physiological measurements; mainly electroencephalography (EEG), electromyography (EMG), and electro-oculography (EOG), which record electrical signals of the brain, as well as muscle and eye activity. Using specialized software, traces from these signals are analyzed and scored visually using characteristic patterns to determine sleep stages according to defined criteria. REM sleep is a deep sleep stage, where the brain is active, the muscle tone is low and there are often frequent eye movements. The majority of human total sleep time is spent in NREM sleep, which can be further divided by 'depth' into 3 stages from light—N1 and N2 sleep to deep N3 or slow wave sleep (SWS). SWS is characterized by high amplitude oscillating activity on the EEG accompanied by lower muscle tone and lack of eye movements. Many of the restorative functions of sleep are thought to occur in this stage[9].

Model	AUC (%)	CA (%)	F1	Precision (%)	Recall (%)
Neural network	92.5	82.3	0.814	81.5	82.3
Random forest	92.6	82.1	0.805	81.3	82.1

Table 1. Overall performance of the neural network and random forest models across all sleeping stages (average over classes) in terms of area under the curve (AUC), classification accuracy (CA), F1 score, precision, and recall (sensitivity).

Dairy cows have been found to sleep for approximately 3–4 h per day, but only around 30 min of this in REM sleep [10,11]. Therefore, most of the sleep time also consists of NREM sleep stages and it is likely that these stages serve important functions for cows as they do humans.

PSG has recently been used to record sleep in calves[12] and cows[10] in indoor-housed environments. However, it requires a considerable amount of training to habituate the animal to wearing the equipment, and this with intensive handling, delicate and expensive devices, specialized scoring and frequent monitoring, makes PSG impractical for large research projects on cows in uncontrolled environments such as in typical group-housed farms and outdoors on pasture. No recent studies have attempted to record non-invasive PSG of sleep of cows on pasture, probably because of the difficulty in using these instruments with cows let alone in challenging and variable outdoor conditions. An ideal solution would be an alternative method or proxy for PSG, more easily applied in a variety of environments and less intensive than PSG. As cows must lie down to sleep[5], lying posture has been suggested as such a proxy. In calves that spend a lot more time in deep sleep stages, lying with head up and immobile and lying with the head resting on the ground or turned and resting on the flank were found to be able to estimate SWS and REM sleep time respectively[12]. However, these same postures greatly over-estimated total sleep time in adult cows[13] and were unable to accurately detect NREM sleep. Further methods based on accelerometers to collect movement and position data from devices on the head or neck of calves and c ows[14–16] have been developed to predict sleep. However, while these models have shown some success in detecting the tucked lying posture during which most REM sleep occurs, they overestimate total sleep time and lack the ability to distinguish differences between light and deep NREM sleep, as well as wakeful inactivity. Additionally, these methods have only been validated with postural estimates of sleep and not with PSG.

During mammalian sleep, autonomic nervous activity such as heart rate[17–19], respiration rate[20] and body temperature change with sleep stage. Machine learning has been used to develop wearable technology for humans such as smart watches that use heart rate and activity to predict human sleep stages and duration[21]. Therefore, the potential exists to use similar physiological changes to identify different sleep stages in cows. In dairy cows, respiration rate and body temperature can be recorded for long periods of time, but are d ifficult[22] or require invasive internal devices. Heart rate (HR) and heart rate variability (HRV) recording devices are relatively inexpensive and unobtrusive to the cow and can be worn for long periods of t ime[23,24]. Methods using machine learning to predict sleep stage from HR and HRV have been developed recently for humans[19,25], and methods combining HR with other measures such as actigraphy further increase performance for sleep stage identification[26].

We collected HR, lying behaviour and PSG data simultaneously from two groups of cows, housed indoors and on pasture. The aim of this project was to determine if we could accurately differentiate between different stages of light and deep sleep in dairy cows using only HR and neck muscle EMG data, compared to visual scoring of the PSG, and to compare the success of two machine learning algorithms in this task.

Results and discussion

EEG is the recognized ‘gold standard’ to determine sleep stages however, a complicated and painstaking setup is required which makes it prohibitive to use for determining sleep stages in cows. The objective of this study was to determine the efficacy of using heart rate and neck muscle activity to determine cow sleep stages using machine learning. To our knowledge, this is the first study of its kind aimed to detect cow sleep stages using only heart and neck muscle data. Using this data alone, the machine learning models developed were able to predict 82.3% of sleep stages correctly. Classification performance of the machine learning models presented in this paper is similar to Mitsukura et al.[27], which proposed models to detect human sleep stages using only heart rate data. Table 1 shows the values used to compare both machine learning models. The neural network (NN) analysis produced the best overall performance and had an area under the curve (AUC) value of 92.5%. Classification accuracy was 82.3%. precision was 81.5%, recall was 82.3% and F1 score was 0.814. The prediction accuracy of the NN model is just marginally better than that of random forest (RF) which produced 82.1% classification accuracy and a slightly better AUC value of 92.6%. Both neural network and random forest algorithms show the ability to learn reasonably well from the data and discriminate well between various sleep stages.

Table 2 shows the CA and AUC of both models to predict the sleep/wakes stages individually. In terms of AUC, Awake and REM stages were the most accurately detected with a 94% and 92% chance of scoring correctly. The models had slightly more difficulty identifying NREM sleep stages; however, AUC was remained at 90%. Figure 1 shows the ROC curves for the classification of each individual sleep stage by both NN and RF models. Classification accuracy for N3 and REM stages were above 95%, with awake and N1/2 ranging from 85 to 88%. Individually, N3 and light N1/2 sleep were slightly more difficult to predict according to the classification performance of various models in our dataset. As previously discussed, this could be due to errors in sleep scoring from the PSG, however NREM sleep stages are the least different from one another physiologically, so it is possible that there is a significant overlap with other sleep stages in the heart rate and neck muscle activity.

Model	Awake		N1/2		N3		REM	
	AUC (%)	CA (%)	AUC (%)	CA (%)	AUC (%)	CA (%)	AUC (%)	CA (%)
Neural network	94.7	88.4	90.8	85.2	90.2	95.3	92.4	95.8
Random forest	94.4	87.2	91.1	85.5	90.4	95.7	92.3	95.9

Table 2. Performance of both models (neural network and random forest) for individual sleep stages (awake, combined light NREM sleep (N1/2), N3 (SWS) and rapid eye movement sleep (REM)) in terms of area under the receiver operator curve (AUC) and classification accuracy (CA).

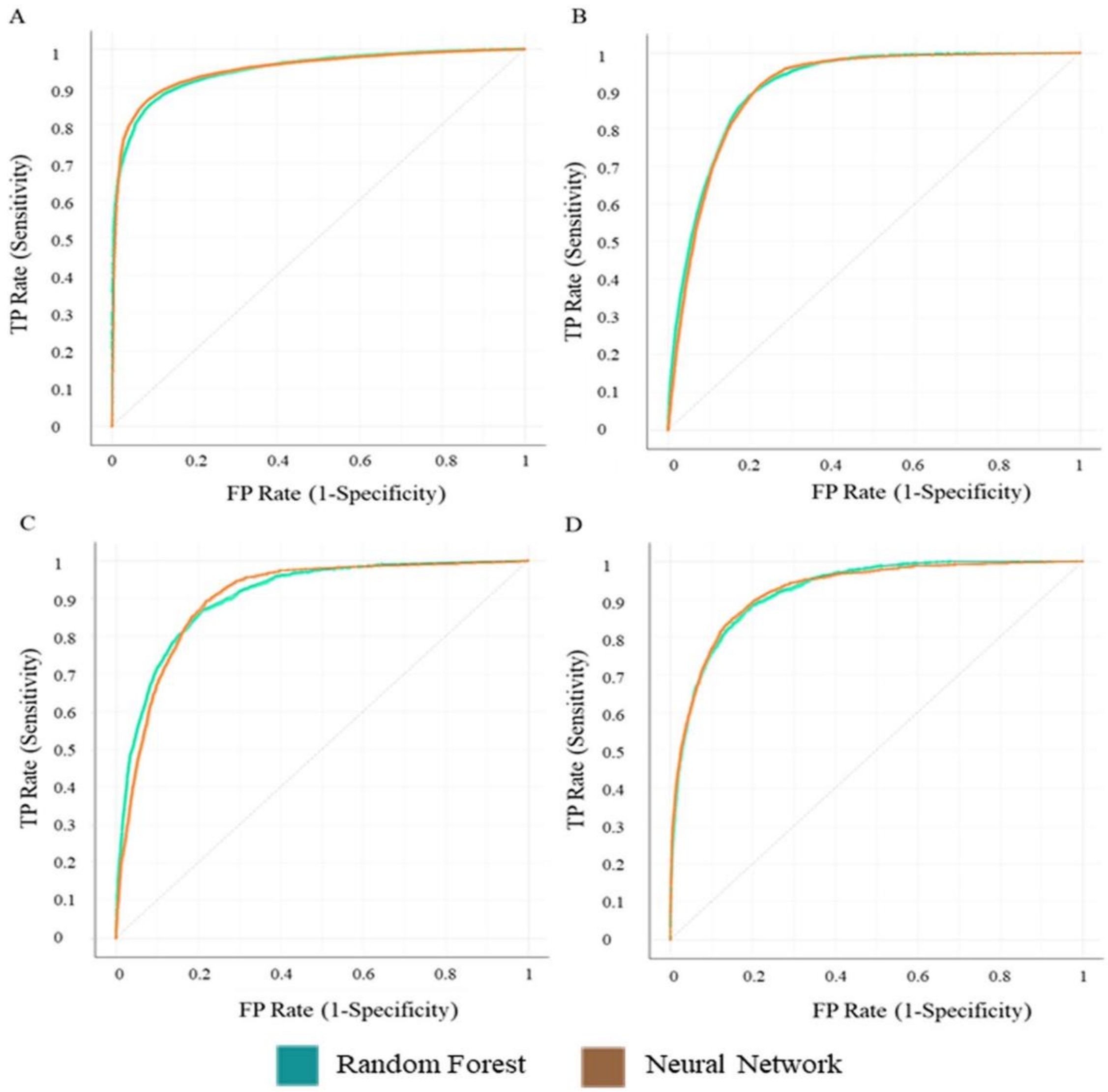


Figure 1. ROC curves of the Neural Network and Random forest models for detection of each individual sleep stage. (**a**) Awake stage, (**b**) combined light sleep stages N1/2, (**c**) slow wave sleep- N3 stage and (**d**) REM sleep stage. Figure created using Orange (version 3.26) https://orang edata mining. com/.

Our methodology involved spending a significant amount of time prior to the beginning of data collection gentling and handling the cows who had previously been unused to such an amount of human contact and training them to wear unfamiliar materials and instruments. Even with these efforts, a large amount of recorded data was then unusable due to cows rubbing electrodes off on gates, water buckets or when lying or moving, unpredictable cow behaviour, or issues with electrode impedance and the devices that could only be determined after the recording. We collected a total of 23,123 useable 30 s epochs (approximately 192 h) of PSG, HR, and activity data from a total of 12 cows in two different environments—housed indoors in the UK and on pasture in New Zealand. As there are no widely used scoring criteria for cows as there are for humans, previous work on

Awake	16,584	71.72%
N1/2	4401	19.03%
N3	1034	4.47%
REM	1104	4.77%
Total	23,123	100%

Table 3. Number of data points and overall percent of data points at each sleep stage in the dataset.

cow sleep[10–12,28] as well as human American Association of Sleep Medicine (AASM)2018 guidelines[29] were used to define sleep stages. Previous cow PSG studies have only identified REM sleep, SWS and 'drowsing', however definitions of drowsing and implications for sleep and cow welfare are unclear[10,30]. Labelling of the sleep stages based on visual analysis of the PSG traces is accepted as common practice in human sleep scoring, however, it can be somewhat subjective and there can be a degree of disagreement even between highly experienced human sleep scoring technicians using clearly defined criteria[31]. A study of inter-rater reliability of human sleep using AASM guidelines found an overall agreement of 82.0% and Cohen's kappa = 0.76[32] and a study of intra-expert scoring of spindles from light sleep found agreement of 72% with k = 0.66[33]. These kappa figures suggest high, but not perfect agreement between observers. Overall intra-observer agreement for scoring sleep/awake stages from the PSG traces in this study was 89.42%, however, N1 and N2 were the least reliable as only 32% of epochs were agreed, and 39% of N1 were re-scored as N2. Combining N1 and N2 improved agreement to 91.1%. Despite an 'almost perfect' level of intra-observer reliability[34], even when combining N1 and N2 stages, 8.9% of epochs were disagreed upon when re-scoring PSG. There is therefore a margin of error introduced into the model due to mistakes in scoring and labelling data from the PSG which was used as the 'ground truth' with which to train the model. However, with visual analysis there is always likely to be a degree of human error associated with the scoring.

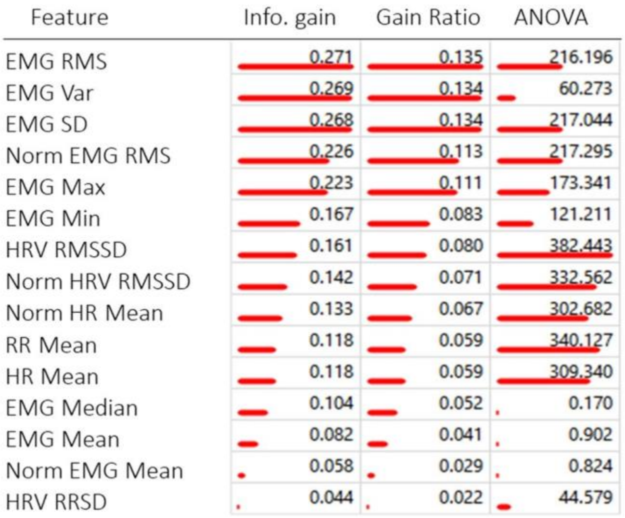
Machine learning has also been used to classify sleep stages in animals such as mice[35] and rats[36] using spectral aspects of the EEG signals, so this could be attempted in future sleep stage labelling of cow PSG data.

Cows are ruminants and must regurgitate and re-chew their food to obtain energy. Because of their strong jaw muscle movements, distinct rhythmic chewing artefacts obscure the PSG traces making accurate identification of any potential sleep stages during rumination or chewing impossible. For this reason, epochs containing rumination were excluded from the dataset and therefore the current model is only able to identify vigilance state from data when rumination is absent. Future models could be modified to predict rumination, however sleep stage estimation during this time would be impacted by the artefacts on the EMG traces.

The data set was heavily weighted to the awake stage. As shown in Table 3, around 70% of the data set consisted of awake data with the other 30% consisting of sleep, and less than 5% of data points being in REM or N3 sleep stages. We made recordings during both day and night and included all recorded data of sufficient quality in the data set. Most cow sleep occurs at night-time, with small bouts of sleep during the day, and in total only about 4 h per day is spent s leeping[11]. Being so heavily weighted to the awake stage, the models had many more examples to learn from to identify Awake epochs, but far fewer examples from which to learn to identify N3 or REM sleep epochs. Balancing the dataset in terms of sleep and awake stages equally might help future models to learn better by having more examples of less common sleep stages.

We used 15 different features of the heart rate and EMG data and the machine learning models were able to learn from this and discriminate between various sleep stages. Classification models learn and perform well when there is a significant difference between features in various classes. Table 4 shows the rank of each feature calculated in terms of information gain (the expected amount of information or entropy), gain ratio (a ratio of the information gain and the attribute's intrinsic information, which reduces the bias towards multivalued features that occurs in information gain) and ANOVA (the difference between average values of the feature in different classes). The features of our dataset that were the most informative for the machine learning models were mainly the Neck EMG features (Neck RMS, Neck Variance, and Neck Standard Deviation). The highest scoring features of our dataset were the Neck EMG features (Neck RMS, Neck Variance, and Neck Standard Deviation). A reduction of muscle tone in the neck muscles is a classical indicator used for the visual identification of REM sleep from PSG data. The higher AUC and accuracy values for the prediction of REM sleep compared to other sleep stages may be due to the high rank of the neck EMG features (Table 2). Mitsukura et al.[27] predominantly used frequency domain features of the HRV signal for sleep stage classification in humans, and it is possible that frequency domain features could be useful for cow sleep staging as well. However, we only used time domain features of the HRV as we were working from 30 s epochs, which is arguably too short of a window to calculate frequency domain metrics from. Frequency metrics are usually calculated for 5 min periods, and while it could be possible to increase epoch size to 5 min to allow for these calculations, this would reduce the granularity and possibly result in longer epochs containing several sleep stages as some bouts of individual stages have durations of less than 2 min. Long epochs consisting of multiple stages could also introduce confusion into the model resulting in more misclassification.

The classification models were developed with data from two separate groups of cows which were different in terms of breed, age, housing, and previous experience. All cows were non-lactating, but the Kiwi-cross (NZ) cows were also in mid-late pregnancy during the recording period. There were differences between the two populations in terms of average HR and the Kiwi-cross cows generally had a higher heart rate than the UK group. These differences could be due to age, size of cows and pregnancy status, but highlights the possibility of hidden batch



Feature	Info. gain	Gain Ratio	ANOVA
EMG RMS	0.271	0.135	216.196
EMG Var	0.269	0.134	60.273
EMG SD	0.268	0.134	217.044
Norm EMG RMS	0.226	0.113	217.295
EMG Max	0.223	0.111	173.341
EMG Min	0.167	0.083	121.211
HRV RMSSD	0.161	0.080	382.443
Norm HRV RMSSD	0.142	0.071	332.562
Norm HR Mean	0.133	0.067	302.682
RR Mean	0.118	0.059	340.127
HR Mean	0.118	0.059	309.340
EMG Median	0.104	0.052	0.170
EMG Mean	0.082	0.041	0.902
Norm EMG Mean	0.058	0.029	0.824
HRV RRSD	0.044	0.022	44.579

Table 4. Ranking of features in the dataset from overall most informative to least and ranking by each calculation; info gain, gain ration and ANOVA (redlines). Table produced using Orange (version 3.26) https:// orangedata mining. com/.

effects within the model. More training data from different populations of cows, and cows in different stages of lactation would be beneficial to increase confidence in the classification ability of the model.

Sleep in mammals typically occurs in cycles with REM sleep following a bout of NREM however, NREM sleep can also occur on its own[37]. Sleep is regulated homeostatically, but achieving a certain amount of REM sleep does not necessarily mean that a proportionate amount of NREM will also be a chieved[37]. In the development of the models, we considered each 30 s epoch independently, however, they are in a time series and make up bouts lasting from a few minutes to a few hours. Preceding epoch classification therefore could have an influence on the classification decision for the successive epoch. Information on typical cow sleep patterns and bout lengths could possibly aid in future models to predict sleep stages in cows.

The current model is a marked improvement over sleep staging models for cows using only accelerometers to predict NREM and REM developed in the past that were only able to predict up to 70% of sleep correctly[14]. These models also used behavioural observations to label sleep stages, which has been shown to overestimate sleep in cows[13]. Our models have been developed with sleep stages labelled using PSG rather than behavioural observations, and while not as simple as accelerometers, EMG and HR monitoring equipment are small and far easier to use with cows than a full PSG montage.

We investigated the use of non-invasively acquired EMG and HR data to predict sleep stages from light N1/2 sleep to deep N3 and REM sleep in dairy cows. While these models have been developed with a small sample size, our classification models developed with Neural Network and Random Forrest algorithms achieved similar outcomes, both with good accuracy, suggesting neck EMG and HR data could be suitable to predict sleep stage with some reliability in dairy cows. More data from cows of different breeds, ages and lactation stages would be beneficial to improve future models. We believe the use of HR and Neck EMG is promising for future identification of sleep stages in dairy cows from non-invasive physiological recording devices. This will enable future research into the effects of typical husbandry practices, transport and environment on cow sleep and the importance of sleep for cow health and welfare.

Methods

Animals and on farm management. Ethical approval for all procedures involving animals was obtained from the UK Home Office (Project Licence P204B097E), SRUC Animal Ethics Committee (Ref. ED AE 03-2018) and Ruakura Animal Ethics committee (AE 14708) prior to study onset. All methods were carried out in accordance with UK and New Zealand animal welfare guidelines and regulations and the authors have complied with the ARRIVE guidelines.

The indoor study was conducted with 6 non-pregnant, Holstein cows (average age 3.86 ± 0.68 years) who were selected from the herd at SRUC Acrehead Farm (Dumfries, Scotland) based on farm staff knowledge of their approachable nature. When enrolled, the cows were either non-lactating or dried off according to routine farm practice prior to the study and were housed in a 20 m × 5 m group pen, deep bedded with straw, within the main barn and fed as per routine farm practice. A 5 m × 5 m test pen was located adjacent to the group pen, but could be separated by a buffer zone of approximately 2 m to reduce potential damage to recording equipment and disruptions to the recordings of other cows, while maintaining visual and auditory contact with the group (Fig. 2).

The protocol was then repeated at pasture with six approachable, mid-late pregnant, non-lactating three-yearold Kiwi-cross (Friesian-Jersey) cows selected from the herd at DairyNZ Lye Farm (Newstead, NZ). These cows were managed outdoors in a large (44 m × 29 m) group pen created with electric fencing that could be moved around within a larger paddock as ground conditions deteriorated. A 10 m × 10 m test pen was created with

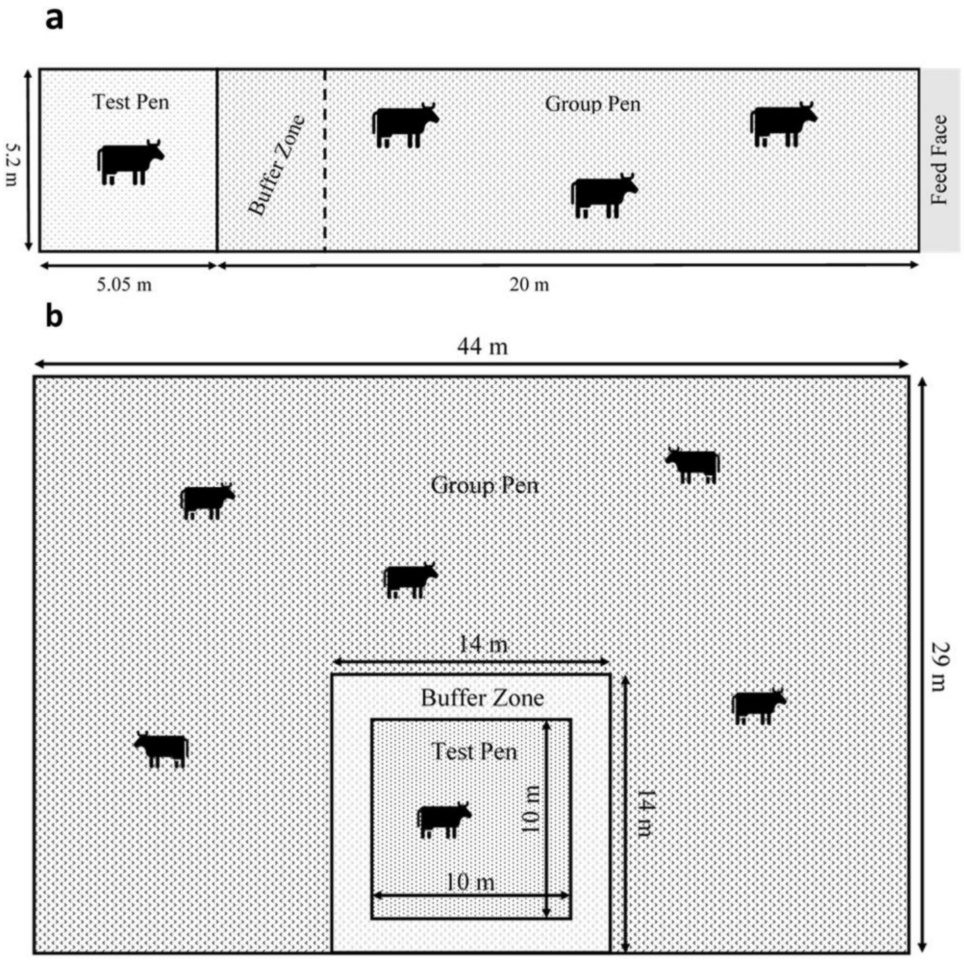


Figure 2. Diagrams of group and test pen design in the UK indoor housed study (**a**) and in the NZ outdoor pasture study (NZ) (**b**). During recordings, the test cow was moved into the test pen, when not recording, the cow was moved back into to the group pen.

non-live electric fencing (to reduce potential electrical noise on physiological traces) on one side of the group pen. A 2 m buffer zone with live electric fencing was set up around the test pen, allowing for visual and auditory contact of the test cow with the group at all times (Fig. 2). Cows were allowed to graze and were supplemented with silage ad libitum. All cows in both groups were trained and habituated to the recording devices and handling protocols for a minimum of 2 weeks prior to the start of data collection.

Data collection methods. *Polysomnography.* PSG was recorded using a 10 electrode montage as described in Hänninen et al.[12]. This included 4 EEG, 2 EOG and 2 EMG electrodes as well as a ground and reference electrode attached to the head and neck of the cow (Fig. 3). Adhesive pre-gelled ECG electrodes (Natus neurology, Kanata, Canada) were used and secured to clipped and cleaned skin on the head and neck of the cow with a small amount of superglue (Loctite 454 or Loctite gel control, Henkel Corp., Dublin, Ireland). A stretchable LeMieux® or Caribu Lycra horse hood (UK: Horse Health Wessex, Woodington, UK. NZ: Caribu AU, Truganina, Australia) was modified for the cow anatomy and worn on the head and neck over top of the electrodes to keep all wires close to the skin and avoid being tangled in the test pen.

After data collection was completed, all materials were removed and electrodes either came away easily or were gently removed using acetone or aqueous cream to soften the glue. Signals were sampled at 500 Hz and recordings ran for 10 h due to memory capacity of the Embletta MPR PG + ST proxy recording device (Embla, Natus Neurology, Kanata, Canada). The recording device was programmed, and data were downloaded using RemLogic 3.4.3 software (Embla Systems, Kanata, Canada). After downloading, a 50 Hz mains filter was applied to all traces to remove the background noise caused from electrical wires that are present in the environment and can be picked up by the PSG device, in the UK and NZ electrical mains frequencies are both at 50 Hz. EEG traces were high pass and low pass filtered at 0.3 Hz and 30 Hz, EOG traces were filtered at 0.15 Hz and 20 Hz and EMG at 10 Hz. Traces were first inspected for quality, "good" quality recordings included those where impedance was within the acceptable range (> 14 Ω) and at least 2 EEG, 1 EMG and 1 EOG trace remained attached for the entire recording period. "Poor" quality recordings were not scored and occurred when impedance was too high, there was noise on the traces, many artefacts obscured the data or electrodes became detached during the recording. Traces were then scored visually in 30 s epochs into 4 stages of sleep (N1, N2, N3, REM), wakefulness (W) and rumination (RNT) by a single scorer trained in human sleep staging, according to staging criteria developed from previous work on cow sleep[10–12,28] as well as human American Association of Sleep Medicine 2018 guidelines[29].

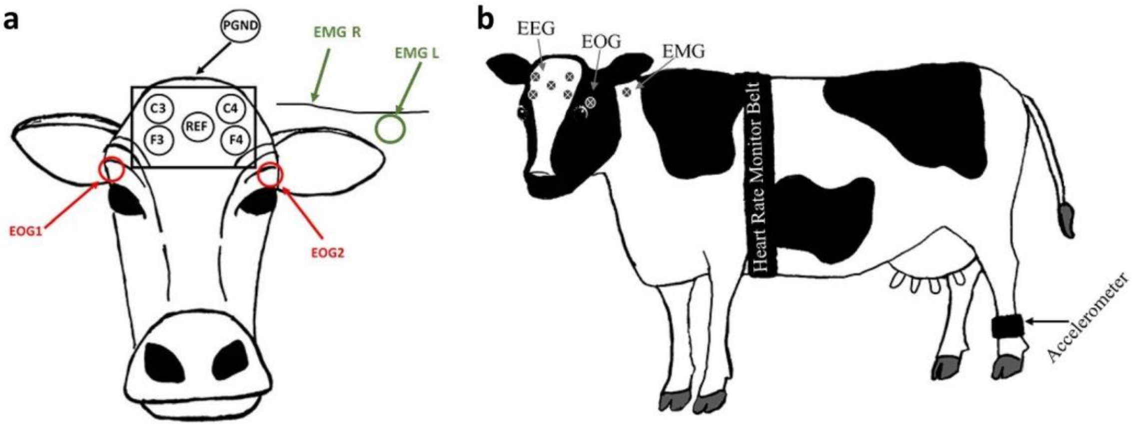


Figure 3. (**a**) Diagram indicating electrode placement on the head and neck of the cow for PSG data acquisition. Four EEG electrodes (C3, C4, F3 & F4) and a reference (REF) electrode were placed on the forehead. PGND- patient grounding electrode was placed behind the poll on the top of the head. Two EOG electrodes were placed beside the eyes and two EMG electrodes were placed on the mid-trapezius muscle on either side of the neck. (**b**) Diagram indicating placement of the heart rate monitoring girth strap, leg mounted accelerometer, and PSG electrodes on the whole cow.

Heart rate. Heart rate (HR) and inter-beat intervals (between R peaks of the heart beat signal: (R-R)) were recorded using a Polar equine monitoring girth strap with electrodes near the heart and the reference electrode near the shoulder (Fig. 3) and logged with the Polar RS800CX Watch (Polar Electro Oy, Kempele, Finland). The time was synchronized between the watch and PSG recording devices. Ultrasound gel (Aquasonic 100 gel, Parker Laboratories, NJ, USA) was applied liberally at electrode locations. Data were downloaded using Polar Pro-Trainer 5 software (Polar Electro Oy, Finland). After downloading, the signal was filtered using Polar Protrainer 5 at a moderate filter power with a minimum protection zone of 6 bpm. Only traces containing less than 1% identified errors were used for analysis. The filtered data were then extracted, and statistics were calculated in 30 s epochs corresponding to the timestamps of the PSG epochs. Only the time domain metrics of the heart rate variability were calculated, as the validity of frequency domain metrics in intervals smaller than the 5 min standard are questionable[38].

Lying behaviour. Lying and standing times were recorded continuously using an accelerometer (UK; IceTags (Ice Robotics, Edinburgh, Scotland), NZ; Onset Pendant G data loggers (64 k, Onset Computer Corporation, Bourne, MA) attached on the lower hind leg (Fig. 3). The data were downloaded using IceManager Software (Ice Robotics, Edinburgh, Scotland) or HOBOware Pro software (Onset Corp., Pocasset, MA). Lying and standing behaviour were determined from the data-logger files in 30 s epochs corresponding to the PSG epochs.

Data pre-processing and segmentation. Neck muscle activity data was extracted from a single good quality EMG trace per recording. Statistics were calculated for each epoch, including mean, maximum (max), minimum (min), median (med), standard deviation (SD), variance (Var) and root mean square (RMS) using RemLogic software.

Mean HR, mean R-R interval, Standard deviation of RR intervals (SDRR) and Root Mean Square of Successive Differences (RMSSD) (Eq. 1) were calculated from the exported and filtered polar heart rate data for each 30 s epoch corresponding to the PSG epochs.

$$RMSSD = \sqrt{\frac{1}{N-1}\left(\sum_{i-1}^{N-1}((RR)_{i-1}-(RR)_i)\right)^2} \quad (1)$$

Normalized HR mean, RMSSD, EMG mean, and EMG RMS values were also calculated by dividing the data by the largest point for each individual recording as a way of removing some of the variation between cows and between recordings. All 15 parameters or 'features' from the HR, HRV, EMG and lying behaviour data were merged and matched with the scored sleep stage epochs using R Studio (Version 1.3.959) using time stamps and epoch numbers.

Intra-observer reliability was calculated using Cohen's kappa in the "irr" package in R (Version 4.0.2). Overall agreement was 89.4% with k = 0.83however, N1 and N2 were the least reliable as only 32% of epochs were agreed, and 39% were misidentified as N2. In exploration of the physiological data, N1 and N2 were not vastly visually different in terms of mean and variance (Fig. 4), and so were combined into a new stage of light sleep named

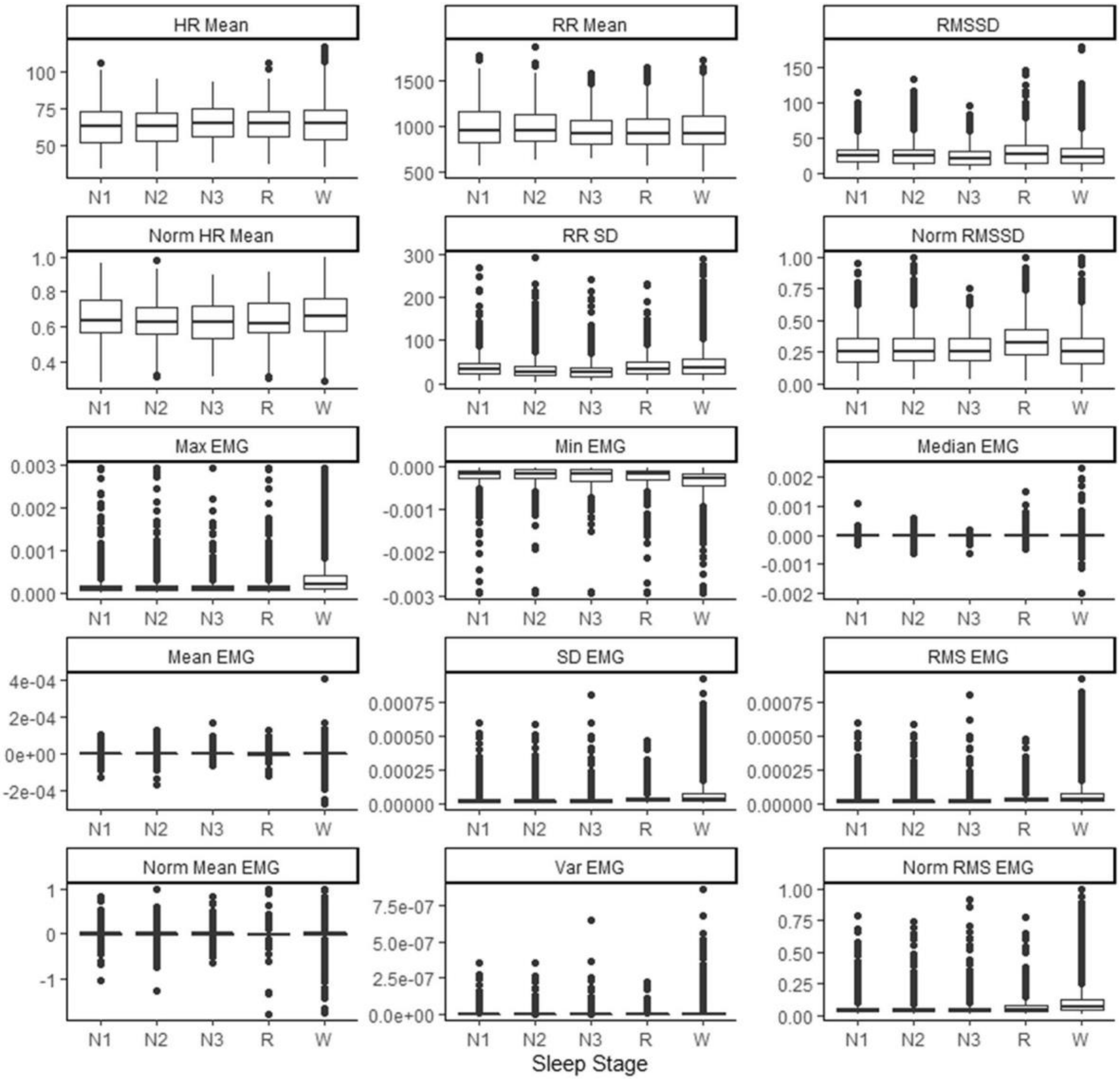


Figure 4. Box and whisker plots of each feature (titles), with sleep stage on the x-axis and relevant units on the y-axis. The y-axis of HR mean, and Norm HR mean are expressed as beats per minute (BPM), while RMSSD, RRSD and the normalized graph of these are expressed in milliseconds. The y-axis of all EMG graphs is expressed in microvolts (µV)Figure produced in R version 4.0.2 using ggplot2 package https:// cran.r- proje ct. org/ web/ packa ges/ ggplo t2/ index. html.

'N1/2'- to improve classification performance. Combination of N1 and N2 improved overall agreement to 91.1% (k = 0.86). Rumination causes rhythmic chewing activity artefacts that obscure the PSG traces and make it impossible to determine brain activity and sleep stage. It is possible that cows could achieve sleep during rumination, which could create confusion and misclassification of the data, so for this reason it was removed from the data set. Dairy cows must lie down to s leep[5], therefore epochs determined as 'standing' from the accelerometer data were also removed from the data set.

From visual and exploratory analysis of the data set, there were no clear differences between sleep stages for any of the features. There were minor differences such as REM sleep tending to have a higher RMSSD than other sleep stages, and the means of W were higher for max EMG, RMS EMG and SD EMG than for the other sleep stages (Fig. 4).

Altogether there were 23,120 data points labelled into 4 different sleep stages (Awake, N1/2, N3 and REM) each with corresponding data from the 15 different features (HR Mean, RR Mean etc.). Table 3 shows number of data points for each sleep stage, the awake category has the greatest number (16,584) of data points, while the combination of N1 and N2 (N1/2) had 4401 data points, REM had 1104 and N3 had 1034 data points.

Machine learning method for sleep stages. To predict cow sleep stages using only heart and neck muscle data, we considered two machine learning techniques: Neural Network[39], and Random Forest[40]. Both the machine learning models were implemented using the open source Orange machine learning platform (Version 3.26)[41]. Stratified tenfold cross-validation was used to train and test the models.

Architecture of the Neural Network Model:

Number of neurons in hidden layers: 500.

Activation function: ReLu.

Solver: Adam.

Regularization: 0.0001.

Maximal number of epochs/iterations: 2000.

During the cross-validation process, the whole dataset was randomly split into a labelled or 'known sleep stage' data set to train the model with, the remaining data having the labels hidden and used to test the model with. For example, REM had 1104 observations, approximately 110 observations were used for testing and rest were used for training and this process was repeated 10 times for each sleep stage. The model's predictions were then compared with the actual labelled sleep stages to test and compare the models. Classification accuracy (CA) (the number of correct predictions divided by the total number of predictions), recall (sensitivity or true positive rate), precision (a measure of the model's exactness), F1 score (the balance between Precision and Recall) and area under the curve (AUC) determined from the receiver operator curve (ROC) values from each model were used to measure the performance. The classification accuracy (Eq. 2), precision (Eq. 3), recall (Eq. 4) and F1 score (Eq. 5) were obtained from true negative (TN), false negative (FN), true positive (TP), and false positive (FP) values. This process was repeated for 10 random splits or 'folds' and classification accuracy of each machine learning technique was measured by taking the average across the 10 folds.

$$Classification\ Accuracy\ (CA) = \frac{TP + TN}{TP + TN + FP + FN} \tag{2}$$

$$Precision = \frac{TP}{TP + FP} \tag{3}$$

$$TP$$

$$Recall = \frac{\quad}{TP + FN} \tag{4}$$

$$F1\ Score = \frac{2TP}{2TP + FP + FN} \tag{5}$$

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Author contributions

The study was conceived by L.H., M.H., F.L., C.O., J.W. and K.S. The experiments and data collection were run by

L.H. Development and testing of the machine learning models were conducted by A.B. The article was drafted by L.H. and revised by M.H., A.B., C.O., F.L., J.W. and K.S. Drawings were prepared by L.H. All authors gave final approval for publication and agree to be held accountable for this work.

Competing interests

The authors declare no competing interests.

Additional information

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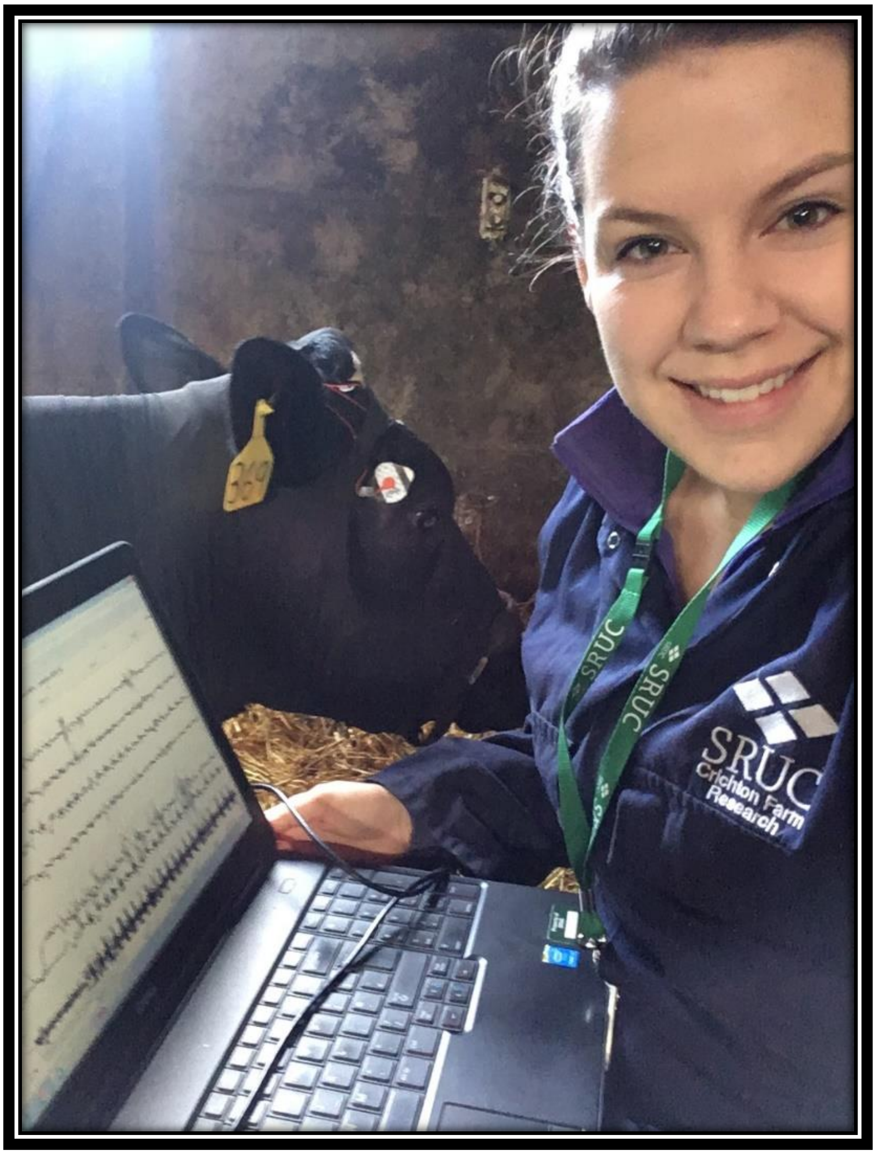
APPENDIX D

Practical Polysomnography Certificate



SleepScience
Sleep Matters

PRACTICAL POLYSOMNOGRAPHY

This is to certify that

Laura Hunter

completed training in full polysomnography including scoring sleep stages, arousals and respiratory events according to AASM standards.

The training was carried out by staff holding the RPSGT certificate
7th – 8th September 2018

'Practical Polysomnography' has been approved by the
Federation of the Royal Colleges of Physicians of the United Kingdom
for **12** category 1 (external) CPD credit(s)
Event code: 120558

D M Fairley

Renata Riha RPSGT FRACP MD FRCPE

Donna Fairley RGN