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The effects of two light programs on sleep in dairy cattle

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Sammanfattning

Sömn är livsviktigt. Trots det är det mycket kring sömn som är okänt. Dagens mjölkkor lever ett stressigt liv, deras höga mjölkavkastning kräver ett stort födointag och långa idisslingstider. Hinner de sova tillräckligt? Vilka faktorer som påverkar mjölkkors sömnbehov är i mångt och mycket okänt. I denna studie studeras ljusets inverkan på sömntid och stå- och liggtider. Fem dräktiga mjölkkor i två grupper utsattes under 36 timmar antingen för artificiellt ljus dygnet runt (ljust) eller artificiellt ljus endast 4 timmar (mörkt) per dygn med en två veckor lång viloperiod mellan behandlingarna. Med hjälp av elektroder fastlimmade på kornas huvuden kunde sömntider och sömnstadium registreras via en EEG-mätare. Stå- och liggtider registrerades via en aktivitetsmätare fastsatt på kons ena bakben.

Denna studie visade att ljus inte verkar ha någon stor effekt på mjölkkors sömn. Möjligtvis hade andra resultat uppnåtts om studien pågått under en längre tid. Vid ljus dygnet var den sammanlagda sov och dåsningstiden signifikant längre tid på dagen än på natten, och under den mörka behandlingen dåsade de signifikant längre under dagen. Dessa resultat är i linje med de få studier som tidigare är gjorda på ämnet. Det fanns inte heller några skillnader i stå- och liggtider mellan behandlingarna. .

Abstract

Sleep is a vital part of life. Even so a lot of the mechanics regarding sleep is unknown. The dairy cattle of today live a stressful life, as their high milk yields forces them to eat large amounts of feed and spend a great portion of the day ruminating. Do they have time to get enough sleep? Which factors affecting the need of sleep in dairy cattle are largely unknown. In this study the effects of light are investigated. A crossover design with five pregnant, lactating cows divided into two groups were subjected to a 36 h period of either complete light or complete darkness (except for a 4 h long lit period per 24 h) with two weeks between each treatment. With glue-on electrodes on the cows heads the sleeping times and sleep stages could be recorded by means of an ambulatory EEG device. Standing and lying times were recorded by an activity meter fastened on one of the hind legs.

The study showed that light seems to have little effect on the sleep in dairy cattle. Other results might have been found if the study had been going on for a longer period of time. The total time spent sleeping and drowsing were slightly higher during the day when compared to the night during the completely lit treatment and more time was also spent drowsing during the day than during the night. These results are in line with previous findings on the subject. There were also no differences in the standing and lying times.

Table of content

1 Introduction

Studies regarding sleep in cattle have during the last years been few and far apart. It is known that cattle are highly motivated to lie down and spend a lot of their time doing so. However, how much cattle need to sleep is not as much researched. Ruckebusch (1972) who investigated sleeping times of three cows, found the total sleep time to be four hs per 24 h. There are several factors affecting sleep time in mammals, but right now it is relatively unknown how light affects sleep times in dairy cattle. In Swedish animal welfare legislation (SJVFS 2010:15) it is specified that dairy cattle must have lights on during the night (allegedly to prevent trampling damage). How the constant illumination affects the cows sleep is unknown. Cattle that in a study were trained to turn the light on and off showed no preferences to sleep or ruminate in the dark rather than in the light, but a weak preference to eat in lit environments (Phillips and Arab, 1998). The aim of the present study is to document the effects of two different light programs (20 h or 4 h of light per 24 h) on the sleep in lactating, pregnant dairy cattle.

2 Literature review

2.1 Sleep and sleep recording

Electrical activity in the brain can be measured using electrodes fastened to the scalp. These recordings are called electroencephalograms (EEG). With EEG recordings it has been shown that sleep in animals can be divided into two stages based on the EEG-patterns; non rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep (Ruckebusch, 1972). NREM sleep is in polygraphic recordings seen as a synchronized high voltage, slow activity pattern with reduced muscle tone. From this stage, animals progress into REM sleep. This is characterized as desynchronized low voltage fast activity patterns and a complete loss of muscle tone. Due to this, during REM sleep in all ruminants, rumen motility and rumination stop (Ruckebusch, 1972). The loss of muscle tone compels the cattle to lie down and have support for the head during REM sleep. It is therefore possible to cause selective sleep deprivation in cattle by keeping them standing, as shown by Ruckebusch (1974b). Even though they can enter NREM sleep whilst standing, they rarely do so. Instead, they prefer to sleep in a recumbent position with their head resting on the ground or on their body. In general, average heart and respiratory rate slows down during transition from wakefulness to sleep (Ruckebusch, 1972). Ruckebusch (1972) also found that sleep generally occurred during two or three periods during the night, with three or four transitions from NREM to REM sleep per sleeping period.

Using only behavioural data it can be hard to tell apart cows sleeping from cows resting or drowsing. Ways to tell them apart without using EEG equipment is to look at rumination (jaw movements), rumen motility and head position which have been shown to correlate with EEG data (Ruckebusch, 1974a). Hokkanen et al (2011) have developed a non-invasive method to predict sleep duration in calves,

using an accelerometer system which has been proven to correctly predict up to 80 % of the sleep duration. One reason that sleep research has been uncommon may be due to the invasive and complicated procedures earlier required for EEG measurements. In the case of for example Ruckebusch (1972), cows had to be implanted with screws drilled into the skull. Today, we can use electrodes fastened to the skin on the animal's head, which is a much easier and non-invasive method (Ternman et al, 2012).

2.2 Sleep deprivation

The high yielding cattle of today spend a large part of the day eating and ruminating to keep up with the high production levels. Especially during the transition period (the time between late pregnancy and early lactation) the nutrient requirements increase significantly (Drackley et al, 2005). After the transition period (to cover up the increase in nutrient requirement) the cow's feed intake is at its highest, making the cow's time budget to be very cramped.

In cattle and other large mammals selective sleep deprivation (in this case REM-sleep deprivation) can be caused by preventing the animals from lying down, as the animals are unable to enter REM sleep while standing up. Ruckebusch (1974b) did a study in which dry cows were hindered from lying down (and thus entering REM sleep) between 14 and 22 hours per day for eight consecutive weeks. It took the cows five to six days to adapt to the 14 h deprivation, and when the deprivation was increased the cows compensated the missed REM sleep by increasing the time spent in NREM sleep. Studies in rats report several severe health problems as an effect of prolonged sleep deprivation. Discovered symptoms include weight loss, increased feed intake, stomach ulcers, increased metabolic rate, impaired immune system and death (Rechtschaffen et al, 1983; Rechtschaffen and Bergmann, 1995; Everson, 1993).

2.3 Effects and implications of increased light

A study on lying behavior in bulls under constant or 12 h of light found that light during the night increased the length of the standing bouts at night, but shortened them during the day. Total lying time differed between the trials with only 3 % (Nicks et al, 1988).

Additional light and longer light periods (16 h of 114-207 lux compared to 9 or 12 h of 39-93 lux) has been found to increase both growth and milk yield in cattle without any increase in consumption of feed (Peters et al, 1978).

Forbes (1982) concludes in a review that increased light periods seem to have a larger effect on the growth of deer and sheep than on cattle. In deer and sheep increased length of the light period stimulated voluntary feed intake which in turn might have affected the growth rates. However, it is not clear whether the increased feed intake was the cause of the increased growth, or an effect of it.

3 Material and method

3.1 Animals and housing

In total five cows were used, divided into two groups (two in group 1, three in group 2). The groups and the treatment schedule can be seen in table 1. All cows were lactating, approximately 4 months pregnant and of the Swedish Red breed. The cows were kept at the Swedish Livestock Research Centre close to Uppsala.

Table 1. Illustrating the order of cow groups and treatments

Week	1 (29/2-2/2)	2 (7/3-9/3)	3	4 (21/3-23/3)	5 (28/3-30/3)
Cow group	1	2	-	1	2
Treatment	Light	Dark	-	Dark	Light

All cows were individually housed in boxes (3x3 m) with straw and peat as litter. Milking took place on set times with a 12 h interval. Milk yield was recorded during every milking and milk samples were collected as part of another study. Silage was provided ad lib and refilled with a 6 h interval. Individual amount of concentrate based on production level was given every 12 h. Water was provided in automatic water cups. Boxes were cleaned manually several times a day. All animal handling and experimental procedures was beforehand approved by the local Uppsala Ethics Committee (ref. C 322/10).

3.2 Recording equipment

All cows were outfitted with electrodes (Unilect, Unomedical Ltd, Stonehouse, Great Britain) (in total 9 per cow) placed on the head and neck to monitor EEG waves, eye movements (using electrooculography, EOG) and muscle tone (elec-

tromyography, EMG). The places where the electrodes adhere were first shaved with trimmer and razor and then cleaned with 70 % alcohol. Electrodes were then glued on using tissue glue (3M VetBond, 3M Animal Care Products, St. Paul, USA). Electrode placement can be seen in figure 1. To process the information from the electrodes, ambulatory EEG recorders (Embla titanium, Embla Systems, Broomfield, USA) were used. The cows were outfitted with an udder holder on which the recording device was fastened. Data from the EEG device were sent via Bluetooth to computers were the information could be tracked live using the computer software RemLogic (RemLogic 2.0.1, Embla Systems, Broomfield, USA). The cows were monitored during the study from an adjacent room using cameras.

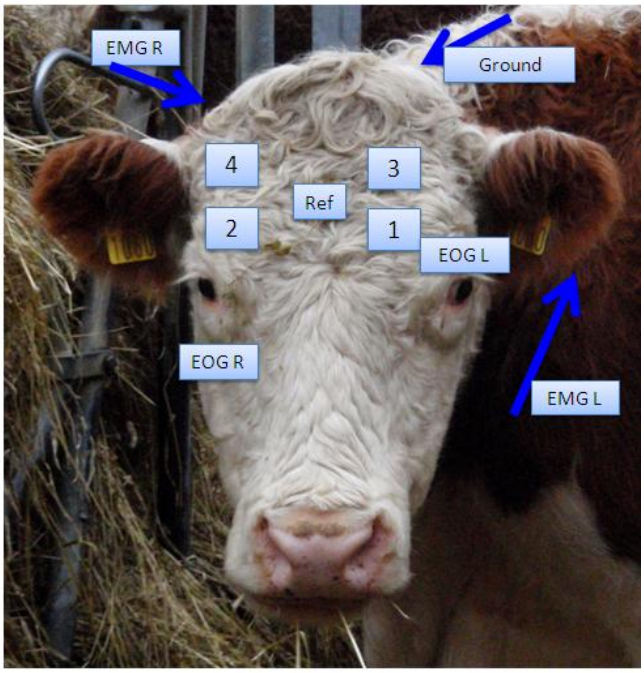

Figure 1. Figure showing the placement of electrodes on the cows head for EEG-monitoring. Picture by Emma Ternman.

3.3 EEG recordings

The cows were taken into the research stable on the Sunday to habituate. They were also outfitted with the udder holders to get used to them. On Tuesday, the cows had permanent catheters inserted into the jugular vein, as part of another study to enable multiple blood samples to be taken. On Wednesday at 10.00 the cows were prepared (shaved) for the fastening of electrodes (for detailed descrip-

tion see Ternman et al, 2012), and in the afternoon at 16.00 the sleep monitoring started, and lasted until Friday at 08.00. A schedule of the research weeks can be seen in table 2. As part of another study regarding the endocrinology of sleep, blood samples were taken hourly between Wednesday at 16.00 and Friday at 8.00. Samples were also collected if the cows fell asleep between the hourly sampling, but only two times per 12 h and sleep stage, and during milking.

Starting on the Sunday when the cows were taken to the stable, the stable was either lit 24 h per day (in this paper referred to as light) or 4 h per day (referred to as dark). During the dark period small red LED lights were lit 24 h per day to ensure some visibility. During the dark weeks full light were on for 4 h between 09.30 and 13.30.

3.4 Recording of standing and lying behavior

All cows carried an activity meter (IceTag3D, IceRobotics Ltd, Edinburgh, Scotland, Great Britain) to continually measure time spent standing up and lying down. The meter was fastened to the left hind leg.

Table 2. The weekly schedule during the experiment

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
05.00		Milking and feeding	Milking and feeding	Milking and feeding	Milking and feeding	Milking and feeding
08.00			Catheterization			End, EEG-recording
10.00				Preparation for electrodes		
11.00		Feeding	Feeding	Feeding	Feeding	
16.00	Cows moved to research stable			Start, EEG-recording		
17.00		Milking and feeding	Milking and feeding	Milking and feeding	Milking and feeding	
23.00		Feeding	Feeding	Feeding	Feeding	

3.5 Data analysis

Sleep time and sleep stages were calculated and scored manually after the experiment ended using data from RemLogic. Before scoring, signals were filtered with the thresholds 0.3-30 Hz for EEG, >10 hz for EMG and 0.15-15 Hz for EOG. The scales for the signals were fixed and scoring was made in 30 second intervals. Power spectrum was used to make the visual scoring easier. The states scored were awake, ruminating, drowsing, NREM sleep and REM sleep. To differentiate between NREM sleep and drowsing, it was decided that activity under 200 nV^2/Hz combined with a low muscle tone was scored as NREM sleep. The data was scored according to Rechtschaffen and Kales (1968) scoring manual for human sleep scoring. By summarizing the total REM and NREM sleep times the total sleep time was obtained, and the total resting time was obtained by adding the total time spent drowsing to the total sleep time.

3.6 Statistics

The data were compared using paired Student's t-tests in Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA). Comparisons were made for each cow between the total sleep time (REM sleep time + NREM sleep time), time spent in each sleep stage and total resting time (REM sleep time + NREM sleep time + total drowsing time) during the dark and light periods.

For three of the cows (due to loss of data for the other two cows) the total sleep time, time in each sleep stage and total resting time during the second day and the second night of the research period were compared using Student's t-test in Microsoft Office Excel. The days were previous to the analysis decided to be between 05.00 and 20.00, and the nights were decided to be between 20.00 and 05.00. These splits were made with regards to the milking times.

The standing and lying data from four out of five cows (due to malfunctioning equipment for one cow) was compared using Student's t-test in Microsoft Office Excel.

4 Results

4.1 EEG data

No significant results could be found on the time of the two sleep stages, time spent drowsing or the total sleep and resting times when light was compared to dark periods. However, there was a tendency towards higher amounts of REM sleep during the dark period than during the light (p-value 0.06). All diagrams regarding the comparisons between light and dark can be found in appendix A.

During the light treatment, the total resting time (total REM-sleep time + total NREM-sleep time + total drowsing time) was significantly higher during the day than during the night (p-value<0.05). Drowsing during the day was also significantly higher during the dark treatment (p-value<0.05). Diagrams showing the comparisons between night and day can be found in appendix B.

4.2 Standing and lying data

No significant difference was detected between the total lying and standing times in the two treatments. Diagrams regarding the standing and lying times can be found in appendix C.

5 Discussion

Finding from the present study indicate that light or dark restrictions of less than a week have little effect on sleep times in cattle. These findings are in line with the few previous studies on the subject that only show small effects on the behavior in cattle due to increased or decreased light (Nicks et al, 1988; Phillips and Arab, 1998). Sleep and sleep research are however complex sciences and it is possible that a longer habituation period before the start of the data collection is needed combined with longer data collection periods for the light treatments to have discernible effects.

In the present study several errors occurred primarily during the first week (mostly due to equipment failure) which led to loss of data. Sleep research on cattle is both time consuming and take up a lot of space, and due to this sample groups are often small (in this case a total of five cows). That data is lost in these situations are not ideal as the sample group shrinks drastically.

The results were in some cases divided up into night and day. The split between night and day were made with regards to the milking times, but ideally the night and the day should have been equally long. In this case, the day was 15 h long and the night only 9 h. Studies later on should take this into consideration.

It is also possible that the four h of light during the dark treatment affected the data and the experiment. It may also have negated any effects that a 24 h long dark period could have brought.

Since this study was done in cooperation with another sleep study focusing on the endocrinology of sleep, this study cannot be seen as a quantification of sleep in dairy cattle. The cows were disturbed by blood sampling at least once per hour, why the total sleep durations cannot be trusted to be accurate. Neither can the amounts of sleep bouts, a figure often seen in sleep research.

It should also be noted that all cows in the study were previously used to continuous lighting. It would have been interesting to have a third group of cows in

this experiment which were subjected to a 12 h light and 12 hours of darkness treatment, or perhaps cows not previously used to continuous lighting. That way it would be clearer whether or not the cows from the light treatment lie down extra short or if the cows in the dark treatment lie down an excessive amount.

It would seem that sleep times in dairy cattle remain the same regardless of the amount of light that the cattle are subjected to. This is in line with previous research. However, due to the small sample size and the limited time of the study, it is not possible to draw a clear conclusion. A longer study with a bigger sample size would be preferred to clearly work out the connection between sleep times and light.

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7 Appendix A

Table 1. Percent of time spent in different stages of sleep and rest during the recording time (TST = Total sleep time, TRT = Total resting time)

	Light					Dark				
Cow ID	REM	NREM	Drowsing	TST	TRT	REM	NREM	Drowsing	TST	TRT
1558	3.6 %	3.4 %	6.4 %	7 %	13.4 %	4.4 %	3.6 %	7.9 %	7.9 %	15.8 %
1557	2 %	4.9 %	4.6 %	6.9 %	11.6 %	4.9 %	6.8 %	8.0 %	11.7 %	19.7 %
1563	3.5 %	6.7 %	7.1 %	10.2 %	17.3 %	4.6 %	4.6 %	8.5 %	9.2 %	17.6 %
1545	3.0 %	5.4 %	12.1 %	8.4 %	20.5 %	2.7 %	3.2 %	11.0 %	5.9 %	16.9 %
1565	2.3 %	14.2 %	15.6 %	16.5 %	32.1 %	5.2 %	16.6 %	10.6 %	21.7 %	32.3 %
Total	2.9 %	7,1 %	9.4 %	10 %	19.4 %	4.3 %	6.9 %	9.2 %	11.2 %	20.4 %

Table 2. P-values when comparing the dark and the light treatments

	REM	NREM	Drowsing	TST	TRT
p-value	0.06	0.8	0.8	0.3	0.4

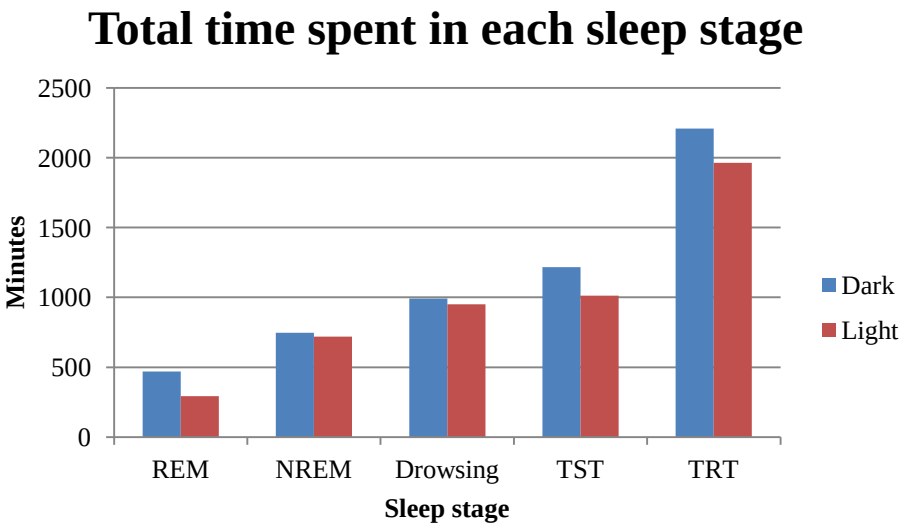

Figure 1. Total times in minutes spent in different sleep stages for five cows during 36 h. Dark = 4 h light/24 h, Light = 24 h/24 h. TST = total sleep time, TRT = total resting time.

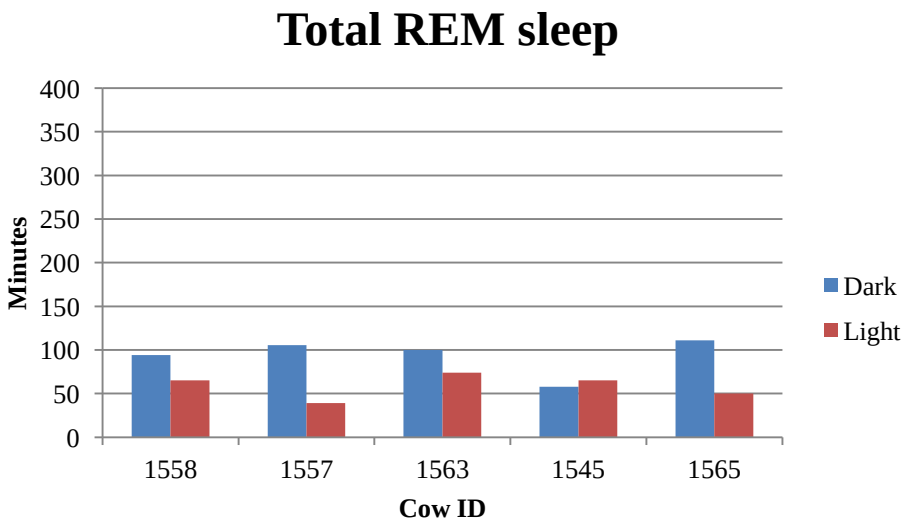

Figure 2. Total time in minutes spent in REM sleep for five cows during 36 h kept in 4 (dark) compared to 24 h light (light)/24 h.

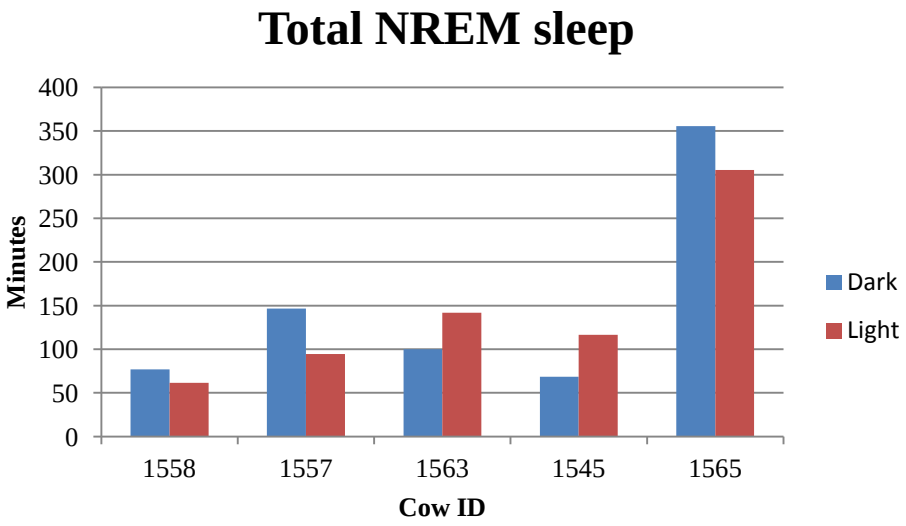

Figure 3. Total time in minutes spent in NREM sleep for five cows during 36 h kept in 4 (dark) compared to 24 h light (light)/24 h.

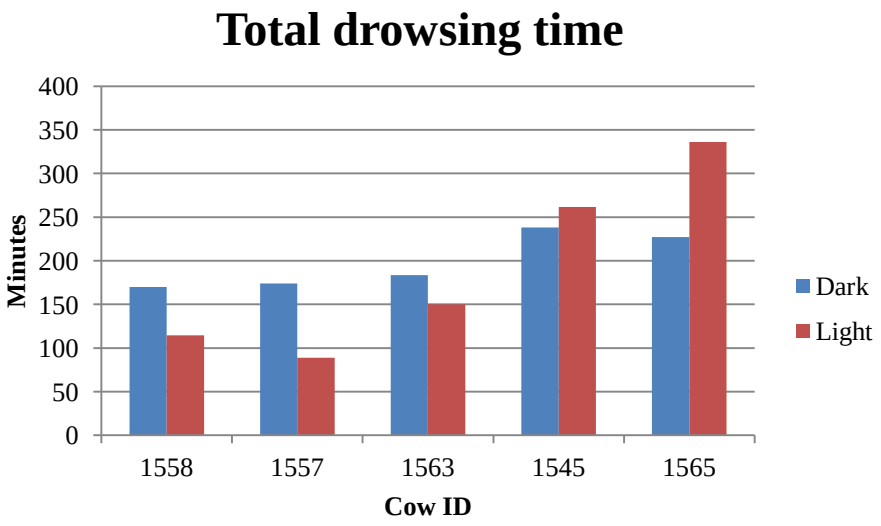

Figure 4. Total time in minutes spent drowsing for five cows during 36 h kept in 4 (dark) compared to 24 h light (light)/24 h.

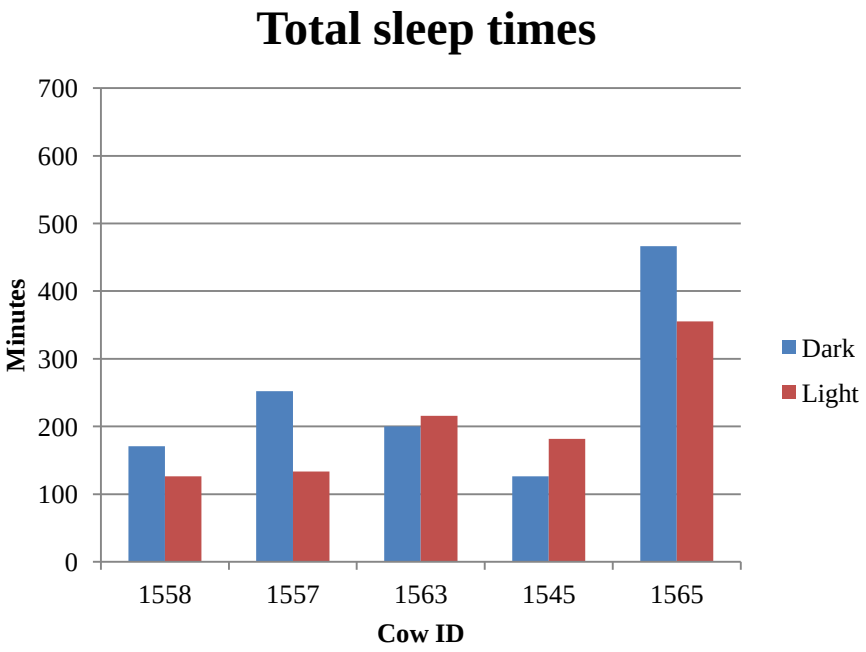

Figure 5. Total sleep times (REM + NREM) in minutes for five cows during 36 h kept in 4 (dark) compared to 24 h light (light)/24 h.

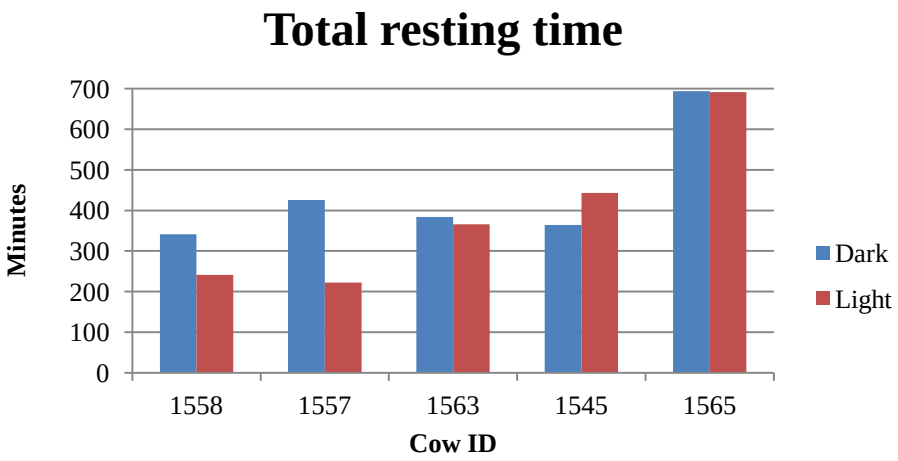

Figure 6. Total resting time (REM + NREM + drowsing) in minutes for five cows during 36 h kept in 4 (dark) compared to 24 h light (light)/24 h.

8 Appendix B

Table 1. P-values when comparing times spent in different sleep stages during the day and night

	REM		NREM		Drowsing		TST		TRT	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
p-value	0.2	0.5	0.1	0.3	0.07	0.02	0.1	0.3	0.03	0.1

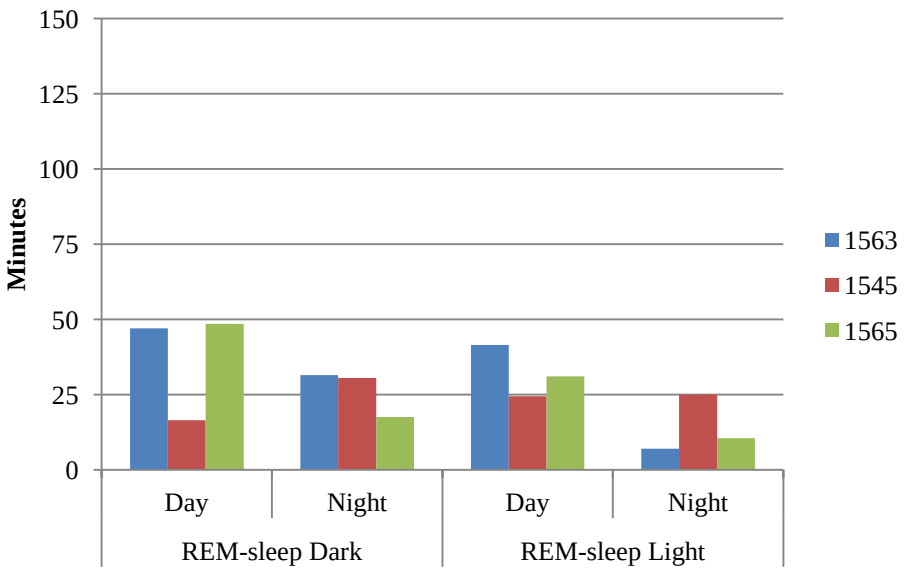

Figure 1. Time in minutes spent in REM sleep during the day (05.00-20.00) and night (20.00-05.00) and during dark (4 h light/24 h) and light (24 h light/24 h) treatments for three cows during 36 h.

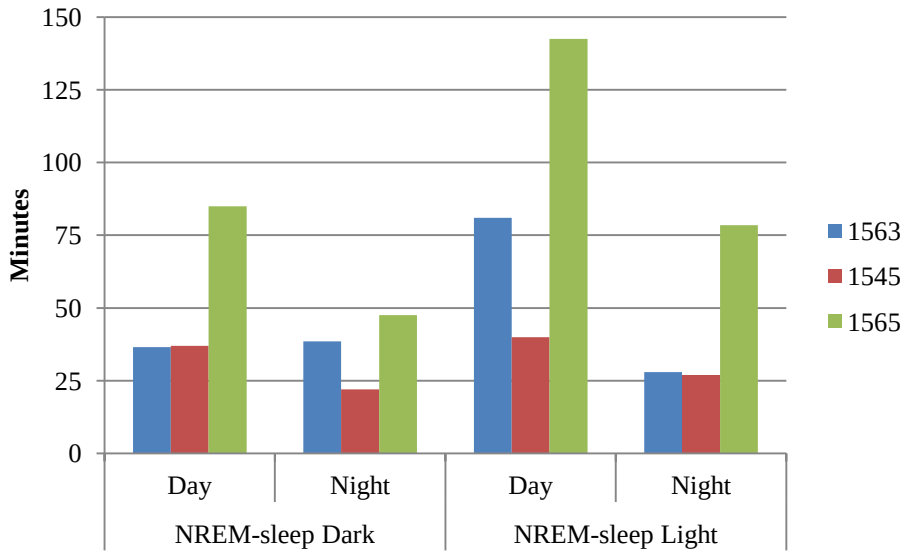

Figure 2. Time (in minutes) spent in NREM sleep during the day (05.00-20.00) and night (20.00-05.00) and during dark (4 h light/24 h) and light (24 h light/24 h) treatments for three cows during 36 h.

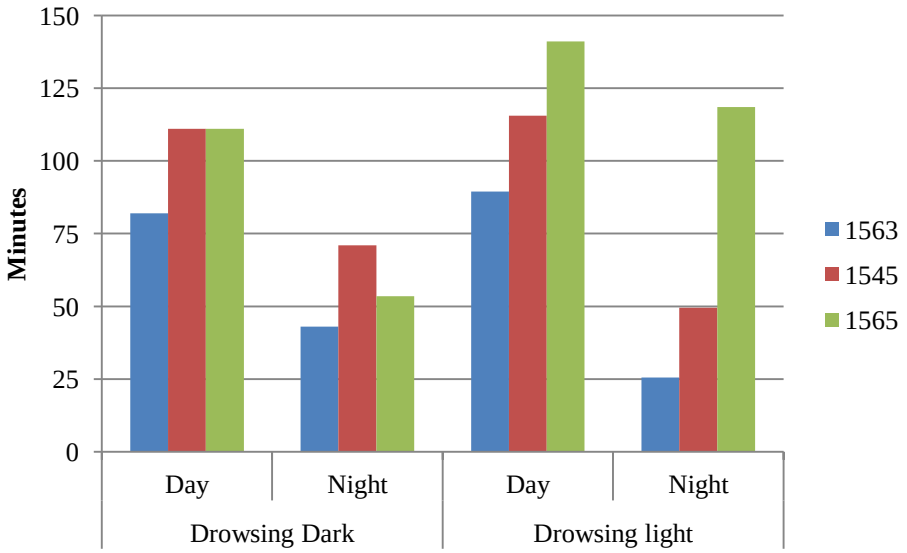

Figure 3. Time (in minutes) spent drowsing during the day (05.00-20.00) and night (20.00-05.00) and during dark (4 h light/24 h) and light (24 h light/24 h) treatments for three cows during 36 h.

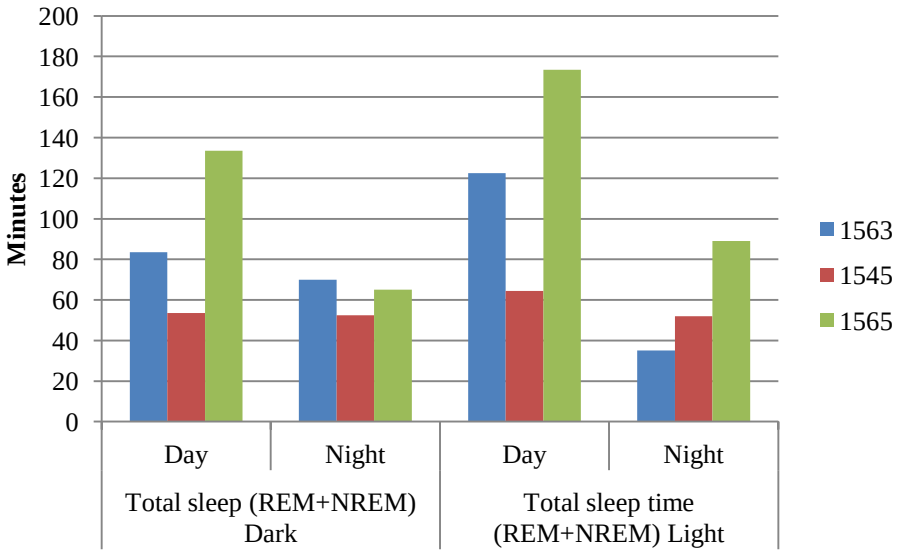

Figure 4. Total sleep time (REM + NREM) (in minutes) during the day (05.00-20.00) and night (20.00-05.00) and during dark (4 h light/24 h) and light (24 h light/24 h) treatments for three cows during 36 h.

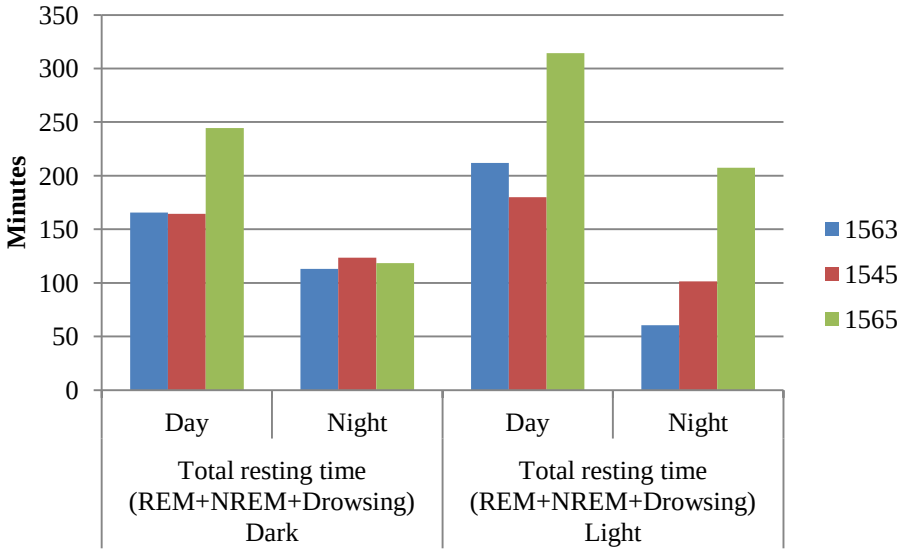

Figure 5. Total resting time (REM + NREM + drowsing) (in minutes) during the day (05.00-20.00) and night (20.00-05.00) and during dark (4 h light/24 h) and light (24 h light/24 h) treatments for three cows during 36 h.

9 Appendix C

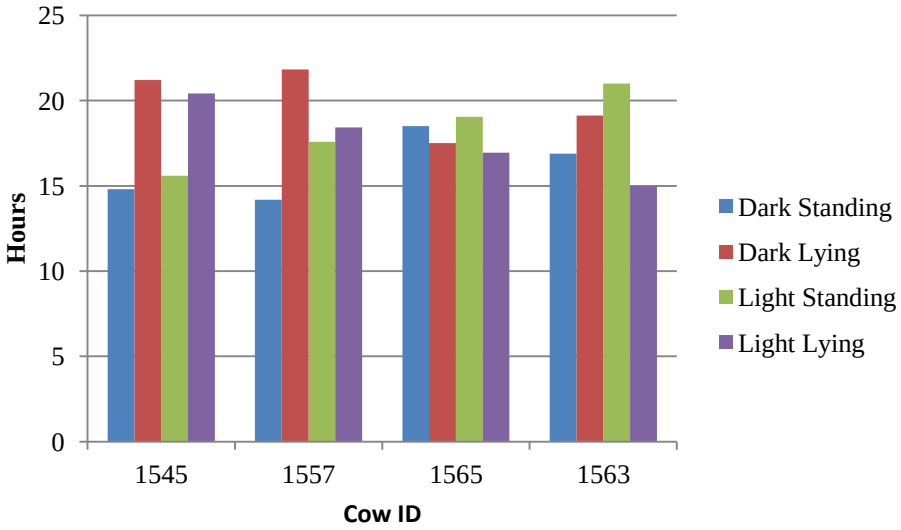

Figure 1. Standing and lying times for five cows during 36 h kept in dark (4 h light/24 h) and light (24 h light/24 h) conditions.

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