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## Comparison between two different transportation schemes

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LIVESTOCK RESEARCH
WAGENINGEN UR

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# Preface

Assessing animal welfare during long distance transport has been a topic of interest for policymakers, competent authorities and the exporting industry. Interpretation of European and National regulations warrants further study for the interest of animal welfare. This report describes how export heifers cope with long distance transport according to two different driving and resting schemes. We expect the results of this study to contribute to evidence supported policy making, preferably at the EU-level.

Dr. ir. Kathalijne Visser

# Summary

Transport conditions for heifers, as well as for other farm animals within the EU, is restricted by EU regulations (EC Regulation 1/2005). Domestic cattle must, after 14 hours of travel, be given a rest period of at least one hour sufficient for them in particular to be given water and if necessary fed. After this rest period, they may be transported for a further 14 hours.
While regulations and training aim to minimize the adverse effects of hazards during loading, transport and unloading of animals, there are, as yet, no recommendations to improve animal welfare that take the circadian rhythm into consideration. Several studies have shown that cattle have distinct diurnal patterns for lying down, eating and ruminating.

This current study aimed to test if the welfare of heifers transported in commercial transports and under Cattle Cruiser conditions[1] was increased if animals were allowed a 9-hour overnight stop (feeding and resting) on the vehicle compared to the common practice in which animals were rested (and fed) at least 1 hour after 14 hours of transport. The results of the study are intended as hypothesis forming and are only applicable to the Cattle Cruiser conditions under which the animals were transported in vehicles with the specifications outlined in Appendix 2.

Behaviour and welfare were monitored continuously during six journeys in the period between June 2013 and June 2014. Average journey length was 1171 km (Woerden, the Netherlands to Sète, France). On each journey one vehicle followed a common practice driving and resting scheme allowing feeding after 14 hours (group A), and the other vehicle followed the longer driving schedule incorporating feeding at the onset of a 9-hour overnight stop on the vehicle (group B). Group A was scheduled to leave at 19:05 hours and to arrive the next day at 13:15 hours; group B was scheduled to leave at 09:00 hours and to arrive the next day at 13:15 hours. Welfare assessment during this transport study was focussed on patterns and duration of lying down and rumination. Each vehicle transported 35 heifers. Per vehicle 8 heifers (2 in each compartment, 4 study compartments per vehicle) were equipped with sensors to register movement (standing and lying down) and to detect eating and ruminating activity continuously. Additionally, heifers were clinically assessed before and after transport, compartment temperature and driving speed were also registered.

Two days before each transport, heifers were equipped with the sensors to collect baseline recordings in the farm environment. Visual inspection of these recordings showed that there were no differences between the heifers transported in group A or B. During transportation the heifers in group B were fed in the evening around 21:00 hours, and had a rest period thereafter until approximately 06:00 hours. Results indicate that the percentage of heifers ruminating increased over this resting period reaching a maximum of 55% 6 hours after the onset of this resting period. The percentage of animals lying down reached a maximum of 35% after 7 hours. While all heifers ruminated at some stage during this 9-hour stop, 4 of the 48 heifers did not lie down during this 9-hour overnight stop. During driving, few animals were lying down (fewer than 15%), with the exception of group B where 30% of the heifers was lying down during the last few hours of the journey. Remarkably, for all 6 journeys combined, 7 heifers in group A and 1 heifer in group B did not lie down at all during the whole journey (from departure until arrival). It has been speculated why these animals did not lie down: a) animals were not feeling ‘safe’ enough to lie down (driving style), b) lack of space and c) the transportation scheme as a whole (short breaks versus longer breaks) was experienced differently for the groups. Nevertheless, although not systematically registered, it was noted that most

[1] See for the Cattle Cruiser concept and specifications Appendix 2

animals from both groups were lying down and ruminating in the collection pens soon after unloading at the destination.

No differences were observed between group A and B in percentages of animals ruminating during driving. In group A, heifers were fed between the third and last driving block, in the morning after 14 hours traveling. Group B heifers were fed before departure, and after the second driving block at the start of the 9-hour overnight stop. Group B (with the 9-hour overnight stop) spent significantly more time eating in the first two hours ($F=20.56$, $p<0.001$), showed a significant increase in percentage of animals lying down ($F=6.59$, $p<0.05$) and rumination ($F=5.57$, $p<0.05$). It is considered that several factors can contribute to this effect: feeding time, cumulative transport duration, and on-farm feeding management prior to departure.

This type of field studies do inevitably involve constraints such as an inability to standardize external factors resulting in difficulties in interpretation of the results. Therefore, the results of the study should be interpreted with care and regarded as hypothetical. Accordingly, the conclusions may be laid down as follows:

The results of the study did not show clear evidence that heifers transported (under Cattle Cruiser conditions) with a 9-hour overnight stop had a profound increase in welfare compared to heifers transported with an one-hour stop after 14 hours of transport. However, it was shown that the 9-hour stop overnight had important advantages for animal welfare:

- After feeding, the rumination activity during the 9-hour resting period on the vehicle was similar (duration and peak) to the rumination pattern in the home environment prior to the journey.

- Providing a stationary environment during the night and during several hours after feeding has a positive effect on both eating duration and rumination pattern. Attention must be paid to provide sufficient space per animal to be able to lie down during the night.

# Samenvatting

Vervoersvoorwaarden voor vaarzen zijn, evenals bij de andere landbouwhuisdieren in de EU, vastgelegd in een EU-transport verordening (Transport Verordening EC 1/2005). Runderen moeten na 14 uur reizen een rusttijd van ten minste een uur gegeven worden om voldoende water en zo nodig voer op te kunnen nemen. Na deze rusttijd kunnen zij voor nog eens 14 uur worden vervoerd.

Terwijl regelgeving en training proberen de nadelige gevolgen tijdens het laden, vervoeren en lossen van dieren zoveel mogelijk te beperken, zijn er, tot op heden, geen aanbevelingen met betrekking tot verbeteren van het welzijn van dieren door rekening te houden met circadiane ritmiek. Verschillende studies hebben laten zien dat runderen een duidelijk circadiaans ritme hebben voor liggen, eten en herkauwen.

In dit onderzoek is het welzijn van fokvaarzen die met de Cattle Cruiser werden vervoerd in een commercieel transport[2] vergeleken tussen een groep dieren die een rustperiode van 9 uur 's nachts op de wagen had en een groep dieren die na 14 uur rijden één uur durende voerpauze op de wagen had. De resultaten van het onderzoek moeten worden geïnterpreteerd als hypothese-vormend en zijn alleen van toepassing op concepten en condities waarin de dieren vervoerd in voertuigen met de vermelde specificaties.

Tussen juni 2013 en juni 2014 is het gedrag van fokvaarzen op zes transporten gedurende het transport continu gemonitord. De totale transport afstand bedroeg 1171 km (Woerden, Nederland naar Sète, Frankrijk). Tijdens elk transport reed één van beide wagens volgens het gebruikelijke schema waarbij de dieren na 14 uur transport gevoerd werden (groep A); de andere wagen volgde het langere schema waarbij de dieren ’s avonds aan het begin van hun rustpauze van 9 uur op de wagen gevoerd werden (groep B). Groep A moest volgens schema om 19:05 uur vertrekken en de volgende dag rond 13:15 uur aankomen; groep B zou volgens schema om 09:00 uur moeten vertrekken en de volgende dag omstreeks 13:15 uur aankomen. Om het welzijn van de dieren tijdens het transport te beoordelen heeft dit onderzoek zich met name gericht op patronen en duur van liggen en herkauwen. Elke wagen transporteerde 35 vaarzen. Per wagen werden 8 vaarzen (2 in elk compartiment, 4 compartimenten per wagen) uitgerust met sensoren om beweging (staan en liggen) en het eten en herkauwen continu te registreren. Daarnaast werden de vaarzen klinisch gecontroleerd op gezondheidsproblemen, en werd de temperatuur in de compartimenten en de rijsnelheid van de wagen geregistreerd.

Twee dagen voorafgaand aan elk transport, werden de vaarzen uitgerust met de sensoren om nulmetingen in de thuis situatie te doen. Uit visuele inspectie van deze nulmetingen bleek dat er geen verschillen waren tussen de groep vaarzen die in de A-of de B-groep getransporteerd zouden worden. Tijdens het transport werden vaarzen in groep B rond 21:00 uur in de avond gevoerd en hadden daarna een rustperiode tot ongeveer 06:00 uur in de ochtend. Resultaten laten zien dat het percentage vaarzen dat herkauwde in deze periode toenam en na 6 uur een maximum van 55% bereikte. Het percentage dieren dat lag bereikte het maximum van 35% na 7 uur. Terwijl alle vaarzen tijdens de 9 uur rustperiode zijn gaan herkauwen, zijn 4 van de 48 vaarzen niet gaan liggen tijdens de 9 uur durende rustperiode. Tijdens het rijden, lagen er een paar dieren (minder dan 15% ), met uitzondering van de vaarzen van groep B waarvan 30% de laatste paar uur van de rit lag. Het was opvallend dat wanneer naar alle 6 de ritten werd gekeken, 7 vaarzen uit groep A en 1 vaars uit groep B tijden het hele transport (van vertrek tot aankomst) niet zijn gaan liggen.. Er kan gespeculeerd worden waarom deze dieren niet zijn gaan liggen: a) de dieren voelden zich niet ‘veilig’ genoeg om te gaan liggen (rijstijl), b) gebrek aan ruimte, c) het hele schema van rij- en rusttijden (korte pauzes versus langere pauzes) voor de groepen is verschillend ervaren. Niettemin, hoewel niet systematisch geregistreerd, werd opgemerkt dat de meeste dieren van beide groepen kort na het lossen in de wachtruimten lagen te herkauwen.

[2] Zie voor het concept van de Cattle Cruiser de specificaties in Appendix 2

Tijdens het rijden waren er geen verschillen tussen groep A en B in de percentages vaarzen dat aan het herkauwen was. In groep A werden de vaarzen gevoerd tussen het derde en het laatste rij-blok, in groep B voor vertrek, en na het tweede rij-blok aan het begin van de 9-uurs rustpauze. Vaarzen uit groep B (met 9 uur rustpauze) hadden een significant hoger percentage dieren dat in de eerste twee uur aan het eten was ($F = 20{,}56$, $p < 0{,}001$), dat ging liggen ($F = 6{,}59$, $p < 0{,}05$) en dat ging herkauwen ($F = 5{,}57$, $p < 0{,}05$) in vergelijking met de dieren uit groep A. Een aantal factoren kunnen hebben bijgedragen aan dit effect: het tijdstip van voeren, de cumulatieve duur van het transport, en het voermanagement voor vertrek.

Dit type praktijkonderzoek brengt onvermijdelijk beperkingen met zich mee zoals het ontbreken van mogelijkheden om externe factoren zoveel als mogelijk te standaardiseren en waardoor de interpretatie van de resultaten bemoeilijkt wordt. Derhalve moeten de resultaten van deze studie voorzichtig worden geïnterpreteerd en moeten de resultaten gezien worden als ‘hypothese-vormend’. De conclusies kunnen daarom als volgt worden geformuleerd:

De resultaten van de studie vormen geen duidelijk bewijs dat fokvaarzen die (onder Cattle Cruiser condities) met een 9 uur durende stop ’s nachts een sterk verbeterd welzijn hebben in vergelijking met fokvaarzen die na 14 uur getransporteerd te zijn 1 uur rustpauze kregen. Echter, het is gebleken dat een 9 uur durende stop ’s nachts belangrijke voordelen had voor dierenwelzijn:

- Na het voeren, was het herkauwpatroon gedurende de 9-uurs periode op de wagen vergelijkbaar (in duur en piek) met het herkauwpatroon op stal voor vertrek

- Het hebben van een stationaire omgeving gedurende de nacht en tot enkele uren na voer tijd heeft positieve effecten op de eet duur en het herkauwpatroon van fokvaarzen. Daarbij moet er aandacht zijn voor voldoende ruimte per dier om ’s nachts te kunnen gaan liggen.

# 1 Introduction

## 1.1 Background

Transport conditions for heifers, as well as for other farm animals within the EU is restricted by EU regulations. Regulation (EC) 1/2005 specifies in Annex I, Chapter V maximum journey times for transportation of certain domestic animals in vehicles equipped according to specifications in Annex I, Chapter VI; after this specified maximum journey time all animals must be unloaded, fed and watered and rested for 24 hours.

Domestic cattle (except unweaned calves which are still on a milk diet) must, after 14 hours of travel, be given a rest period of at least one hour sufficient for them in particular to be given liquid and if necessary fed. After this rest period, which may be extended to a maximum of 3 hours, but only if this is needed for the care of the animals, they may be transported for a further 14 hours (cited from (EC(EuropeanCommision), 2005). The total duration of the journey may therefore take no longer than 31 hours.

The daily driving time allowance for the truck driver is the total accumulated driving time in one day, which is set at 9 hours, but may (twice a week) be extended to 10 hours. Each period may not last longer than 4.5 hour. Thereafter, a break of at least 45 minutes is compulsory (Regulation (EC) 561/20062).

This poses the interesting question how to meet the two different EU regulations without compromising the welfare of either the truck driver or his animals.

## 1.2 Welfare assessment and animal transport

Welfare is clearly a characteristic of an individual animal and is concerned with the effects of all aspects of its genotype and environment on the individual (Duncan, 1981). Broom (1986) defines it as follows: the welfare of an animal is its state as regards its attempts to cope with its environment. In order to safeguard welfare and avoid suffering, a wide range of needs must be fulfilled.

Consideration of the risk/benefit assessment in animal welfare focused on defined welfare outcomes, rather than driven by input factors, is consistent with current thinking as exemplified by the Welfare Quality® project (Botreau et al., 2007). Welfare Quality® projects have provided observations and clinical measurements that can be implemented as practical indicators of welfare hazards associated with transportation of animals. For example, ‘heat stress’ may be assessed by observing the animals sweating and thermal panting. Additionally, measurement  of body temperature can support a sound welfare assessment. However, observance and monitoring of animal welfare indicators should be performed without disturbance to the animal to minimise the stress already attributed to the transport itself (Lambooij et al., 2012).

The use of sensor technology in agriculture has taken enormous steps in the last decade. Data collected by sensors on the farm can be transmitted to a central site for processing, storage and reporting (Banhazi et al., 2012). This has the potential to provide considerable advantages for farm management. In dairy farming, the collection of data using automatic milking systems, has already been proven to be beneficial for the farmer (Hogeveen and Ouweltjes, 2003). Automatic activity monitoring in dairy cattle has been shown to be a useful tool in the detection of lameness (De Mol et al., 2013).

The use of sensor technology to monitor physiology and behaviour during transportation has been used since midst nineties (Ville et al., 1993). Nowadays, technology has been improved and use of sensor technology, also for commercial transporters, is available on the market. For research purposes body temperature (Gerritzen et al., 2013) and heart rate (Gerritzen et al., 2013; Hindle et al., 2013; Lambooij et al., 2012; Munsters et al., 2013; Schmidt et al., 2010) have been used as a physiological indicator of stress and welfare of animals during transport.

On farm, deprivation studies with cattle have shown that patterns, frequencies and duration of lying down, feeding and ruminating can be affected considerably by stress in housing and management. It has been shown that the environment where cattle is housed can affect lying behaviour. For example, the lying time of cows in free stalls is reduced in proportion to the degree of overcrowding (Wierenga and Hopster, 1990). Cows housed in straw yards lie down for longer than those in free stalls (Phillips and Schofield, 1994). It was shown that cattle attempted to compensate for the reduced lying time (Jensen et al., 2004; Ruckebusch, 1974), and others have shown that both feeding and lying deprivation resulted in compensatory behaviour indicating that lying down appeared to have a higher priority than feeding (Metz, 1985; Munksgaard et al., 2005).

Rumination is an essential behaviour in ruminants; it is required to reduce the size of feed particles to facilitate passage through the reticulo-omasal orifice (Welch, 1982). Furthermore, the production of saliva prevents the rumen from acidosis and it is essential in providing rumen bacteria greater access to feed particles during microbial fermentation (Russell and Rychlik, 2001). Cows prefer to ruminate while lying down (Cooper et al., 2007). Phillips and Leaver (1986) reported that cows normally ruminate when lying down for approximately 6 hours a day. Cows only ruminate while standing for 1 hour a day. When cows are prevented from lying down, they increase their time spent ruminating while standing according to increasing deprivation time (Cooper et al., 2007; Munksgaard et al., 1999).

Cyclic patterns in behaviour and physiology resulting from adaptation to the natural light cycles are well established in cattle and easily recognizable, as can be seen from the diurnal rhythm of feeding and rumination of cows housed individually under controlled lighting conditions (EFSA, 2009).

## 1.3 Research question

This current study aimed to test if the welfare of heifers transported in commercial transports and under Cattle Cruiser conditions[3] was increased if animals were allowed a 9-hour overnight stop (feeding and resting) on the vehicle compared to the common practice in which animals were rested (and fed) at least 1 hour after 14 hours of transport.

The results of the study are intended as hypothesis forming and are only applicable to the Cattle Cruiser conditions under which the animals were transported in vehicles with the specifications outlined in Appendix 2.

[3] See for the Cattle Cruiser concept and specifications Appendix 2

# 2 Material and methods

## 2.1 Study Design

The following conditions restricted the set up for the study

- the total journey time should fall within the EU COUNCIL REGULATION (EC) No 1/2005 on the protection of animals during transport and related operations, the permissible daily driving time for each driver is 9 hours
- driving and resting periods should be comparable with common practice
- animal feeding times should be similar to common practice
- arrival time of both vehicles should be, for practical reasons, aligned with unloading times at the harbour, i.e. before 14:00 hours

For the current study one commercial exporter (Van Dommelen BV) allowed their transports to be followed. In order to standardize the journeys a single commercial route was chosen: Woerden (the Netherlands) to Sète (France) (see figure 2.1). The journey was 1171 ± 21 km (depending on traffic diversions). See for more details journey logs provided by the drivers Appendix 1.

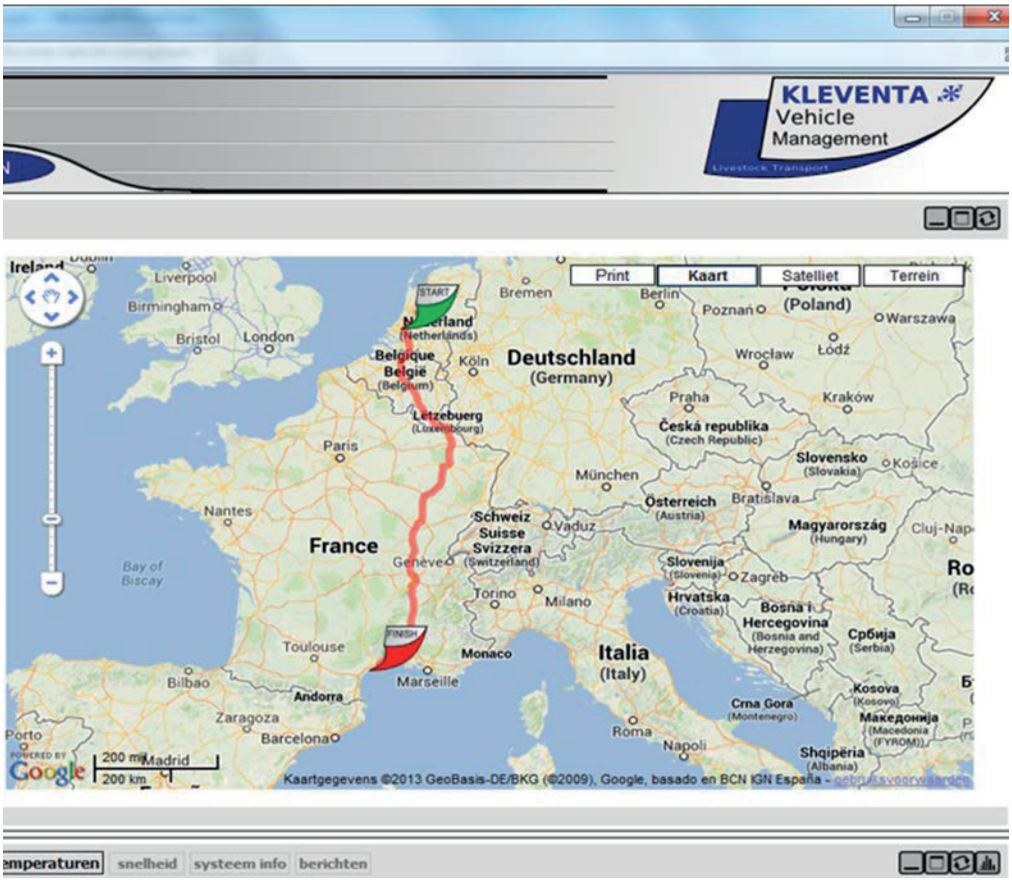


***Figure 2.1*** *Route from Woerden (the Netherlands) to Sète (France)*

Considering the above mentioned conditions, with the help of statisticians the following schedule was designed (see figure 2.2). In treatment A, dairy heifers were transported according to the current EC Regulation 1/2005, with two drivers, only a short break was allowed for feeding the animals after the first

14 hour of the journey. In treatment B, heifers were transported according to an alternative schedule, with one driver, and having the possibility to feed and rest during a 9 hour break at night time.

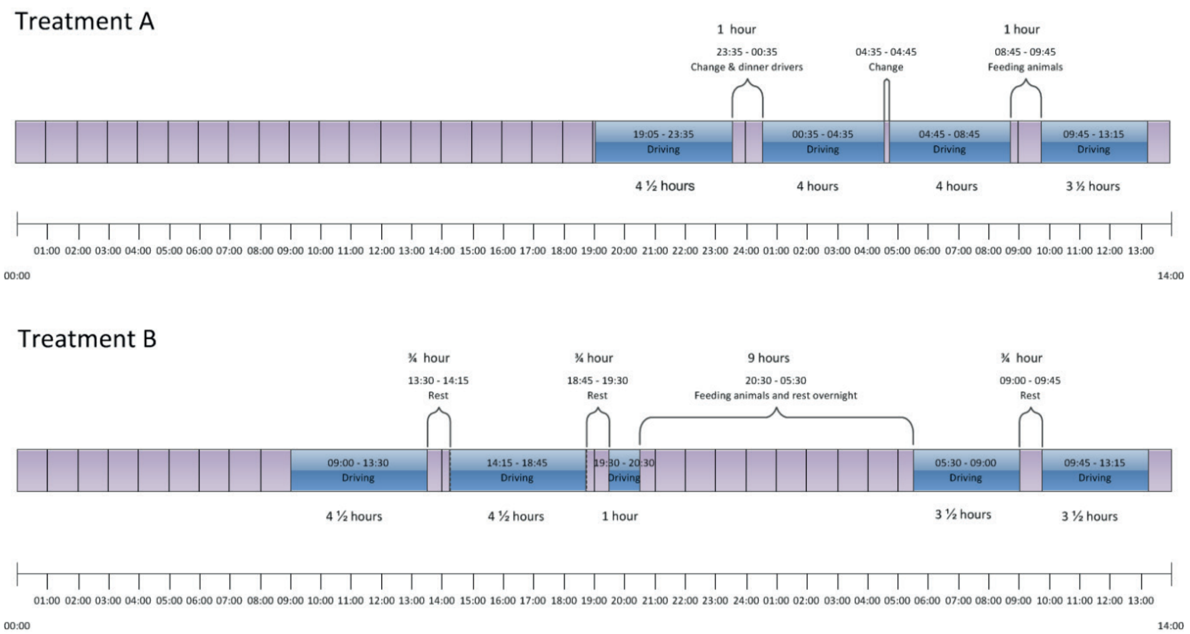


***Figure 2.2*** *Schematic presentation of the driving and resting times for the two treatments (treatment A without 9-hour overnight stop and treatment B with a 9-hour overnight stop)*

The comparison between treatment A en B was studied six times between June 2013 and June 2014 (see Appendix 1). Both treatments were executed at the same time, hence the vehicle with heifers for treatment B departed in the morning and the vehicle with the heifers for treatment A the same day, early in the evening. Both with an expected arrival time of 13:15 the following day.

In the course of the study the departure times were adjusted due to allow for traffic conditions (i.e. expected rush hour congestion) and alignment of arrival times. Therefore from the second journey onwards, the departure time of treatment B was set at 9:00 am (originally 8:00 in journey 1) and for treatment A from the third journey onwards the departure time was 19:05 pm, originally 18:05 pm on the first two journeys.

The experiment was approved (DEC 2013029.b and DEC 2013029c) beforehand by the Ethical Committee of the Animal Sciences Group of Wageningen UR, The Netherlands.

## 2.2 Animals and vehicle

### 2.2.1 Animals

Each vehicle was loaded with 35 dairy heifers for export with an average age of 22.8 ± 2.4 months, were on average 4.9 ± 1.5 months in-calf[4] (maximum 6 months), and weighted on average[5] 545.9 ± 5.2 kg. Heifers were bought from different farms throughout the country and were quarantined for at least 3 weeks before departure at the collection point in Woerden. All animals were placed in smaller groups of 5-8 individuals at least one week before departure. Animals housed in the same group were transported in the same compartment.

Heifers had ad libitum access to water and were fed corn and ad libitum silage during their stay in the quarantine units. Two times a day fresh food was delivered, once in the morning and once in the afternoon. Group B, departing in the morning, received their daily fresh food a few hours before departure, at their normal feeding time (06:00 hours).

### 2.2.2 Vehicle

The vehicles used for this study were of the type Cattle Cruiser (see Appendix 2 for specifications of this vehicle type).

In order to maintain driving stability and safety in spite of the animals' different body weights, different numbers of heifers were assigned to the 5 compartments. Compartment 1 (C1) housed 8 of the smallest (lightest weight) heifers, compartment 2 (C2) 8 heavier heifers, compartment 3 (C3) 7 medium weight heifers, compartment 4 (C4) housed the 7 heaviest heifers and compartment 5 (C5) 5 medium to heavy heifers. The compartment sizes measured 11.1 $m^2$, 10.8 $m^2$, 10.8 $m^2$, 11.4 $m^2$ and 8.6 $m^2$ respectively for compartments C1-C5.

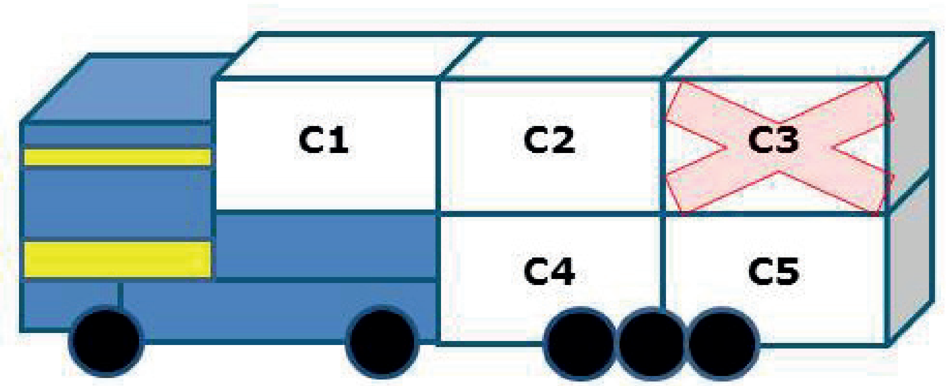


***Figure 2.3*** *Schematic presentation of the compartments on the vehicle*

The floor of compartment 3 (C3) was slightly different from that of the other compartments because of the foot battens allowing animals to climb to the upper level during loading and descend during unloading. In order to avoid the risk that animals in this compartment behaved differently, this compartment was excluded from the study (see figure 2.3).

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[4] In-calf information of the first journey was used

[5] Average weight is calculated as the netto weight of the vehicle divided by 35 (=number of heifers).

***Figure 2.4*** *Inside the vehicle where the bedding consist of a thick layer of straw*


***Figure 2.5*** *Specially designed feeding trunks on the outside of the vehicle to feed the heifers during the break*

During the journey animals had ad libitum access to water in each compartment. Each compartment floor was covered with a thick bedding of straw (see figure 2.4). Animals were fed silage in the feeding trunks specifically designed for this vehicle (see figure 2.5) according to the driving and resting schedule (see figure 2.2). A salt/mineral block was provide in each compartment (see figure 2.9).

## 2.3 Measurements

It was not expected that heifers transported under the conditions specified in Appendix 2 would suffer from extreme heat/cold stress or thirst. Furthermore, with a space allowance per animal of 1.4-1.7 $m^2$ and a compartment height of 180 cm it was not expected that animals would be susceptible to wounding due to lack of space and/or aggressive behaviour.
Therefore, welfare assessment was focussed on behavioural patterns (lying, feeding, ruminating) rather than physiological homeostasis and scoring for injuries or wounding. Nevertheless, a clinical scoring for abnormalities was included in the protocol. Additionally, data-loggers for heart rate registration were used during the first journey to detect possible abnormalities.

Two ‘study’ heifers were chosen at random in each of the four study compartments (C1, C2, C4, C5). These individuals were equipped with sensors for registration of heart rate activity (journey 1), activity (standing and lying down), rumination (and feeding). Additionally, these individuals were clinically scored for health and condition. Compartments C1, C2, C4, C5 were equipped with a video camera and an additional temperature (and relative humidity in compartment 1) sensor.

Baseline recordings for heart rate, activity (lying down and standing) and rumination (and eating) activity were taken for a 24 hour period within the two days preceding the day of departure.

### 2.3.1 Heart rate

In earlier studies, Lambooij et al. (2012) found that heart rate increased in cattle during loading and decreased as transport commenced.
In the first journey heart rate was registered using ECG electrodes. The measuring equipment (data logger) used was specially designed by the Royal Veterinary College in London (Lowe et al., 2007). This data logger was housed in a metal box. In order to secure placement of monitoring equipment a specially designed jacket was made for use in this experiment (see figure 2.6). To equip the study the locations for the electrodes were shaved to secure effective contact between skin and pad electrodes. Before placement of the electrodes the skin was rinsed with water, dried and cleaned with 70% alcohol. Surgical glue was applied to secure the pad electrodes to the surface of the skin. Electrodes were placed caudal to the olecranon on both sides of the breast. The earth electrode being placed dorsally to the electrode on the right side of the breast. The sensor leads were attached to a data logger which was housed in a stainless steel container and secured in a pouch on the back of the animal. Logging started immediately after fitting the data logger and was terminated after being unloaded at the place of destination.
ECG traces were analysed as heart rate in beats per minute (Labchart7 Pro, V7.1.2, AD Instruments, Cologne, Germany).

***Figure 2.6*** *Data loggers housed in a stainless steel container and secured in a pouch on the back of the animal to register ECG continuously*

### 2.3.2 Activity

Activity was recorded using 3-dimensional accelerometers, commonly referred to as pedometers, at 4 Hz (IceQube™, IceRobotics Ltd., South Queensferry, UK). Throughout the study period, each cow was equipped with an IceQube which was attached to the fetlock of the left hind leg (see figure 2.7). IceQubes continuously determined whether a cow was lying down or standing, and recorded the number of steps per 15 minute period. After the study, data from IceQubes were transferred by IceReader interface to a computer equipped with IceManager software. These data were then used to derive the laying and standing bouts of the test cows.

***Figure 2.7*** *IceQube™ attached to the left hind leg to record lying down or standing activity*

### 2.3.3 Rumination and feeding

Monitoring feeding and rumination patterns serves as a crucial and helpful parameter (Devries et al., 2009) in gaining relevant information about the individual animal and its ability to cope with specific feeding, housing, and management situations (Devries et al., 2009; Owens et al., 1998).

Automated sensor based technology was used in order to measure rumination activity and eating activity during transportation. The RumiWatchSystem® (ITIN+HOCH; Ettenhausen, CH) sensor-based system enables automatic measurement of rumination and feed intake (see figure 2.8). It incorporates a noseband sensor, data logger and evaluation software. The sensor registers jaw movements at a frequency of 10 signals per second. The data are saved on a SD Memory Card and transferred to computer with operational software. The system has been validated for monitoring feeding and ruminating behaviour and constitutes a high accuracy for measurement of feeding behaviour of dairy cows and demonstrates respectable concordance of visual and automated measurement of rumination and eating (Zehner et al., 2012). In a recent study of the RumiWatchSystem®, it has been demonstrated that the sensitivity and specificity is provided through differentiability of jaw movement as rumination, eating, drinking or other activities . Each jaw movement corresponds to a specific deflection, allowing an exact allocation (Büchel and Sundrum, submitted).

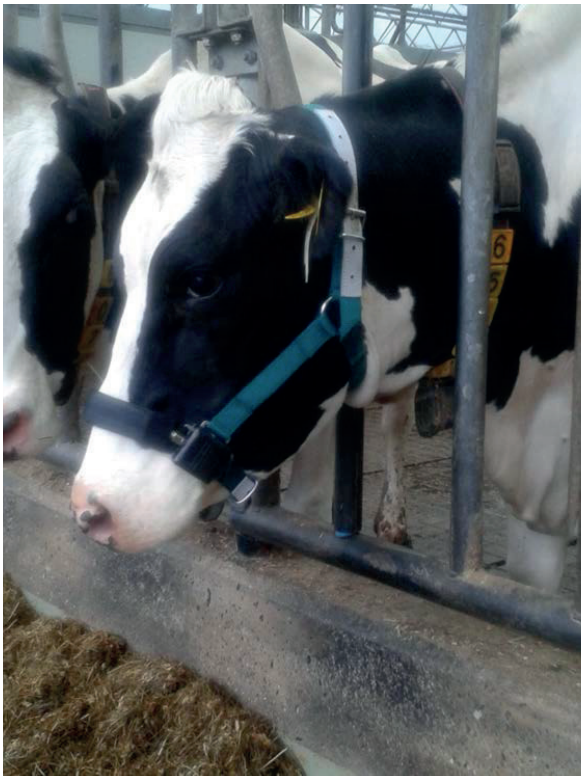

***Figure 2.8*** *A cow fitted with a RumiWatchSystem® halter to register eating and rumination*

### 2.3.4 Water consumption

Water consumption was not measured directly on the animals. The water consumption was an estimate based on the percentage of water that was used (drinking and spilling). Water loss due to sweating and urinating was not taken into account.

### 2.3.5 Clinical condition

Clinical condition of the 8 'study' heifers per vehicle was assessed three times: at the start of the baseline measurement, before loading and immediately after unloading. The same researchers were trained by a veterinarian in clinical qualitative scoring prior to the study.
Clinical assessment of the heifers was based on the following aspects: (see Appendix 3):

- respiratory rate (fast / slow)
- respiration (flat / deep)
- extra sounds during respiration (yes / no)
- short of breath (yes / no)
- rectal temperature
- ear temperature (cold / normal / warm)
- skin lesions (no / number)
- coat (wet / dry)
- cleanliness (dirty / clean)
- claws normal (yes / no)
- mucous membranes (pink / red / pale)
- behaviour (normal / spooky / quiet / aggressive)
- posture normal (yes / no)
- locomotion (lame / sound)
- manure (normal / thick / thin / not visible)
- hindquarters (clean / dirty)
- tail (clean / dirty)
- small scratches (yes/no)

### 2.3.6 Environmental temperature

Temperature sensors were placed in the ceiling of all five compartments of the vehicles and registered compartment temperatures throughout the journey. This system was provided by Kleventa® Vehicle Management. The software enables the driver but also the 'home office' to monitor the compartment temperatures closely. Temperatures were sampled at intervals of approximately 10 minutes. Results of these temperature measurements were kindly provided for this study.

Additionally, ATV-01 data loggers (www.atal.nl; Purmerend, the Netherlands) were attached to the ceiling of the four study compartments (C1, C2, C4, C5). ATV data recorders registered environmental temperature with sampling at intervals of 5 minutes. In C1 the ATV data recorder also recorded relative humidity at 5 minute intervals.

### 2.3.7 Driving speed

The driving speed of the vehicle was registered every 10 minutes by Kleventa® Vehicle Management and kindly provided for this study.

### 2.3.8 Video recordings

All four study compartments (C1, C2, C4, C5) were equipped with a video camera to record the behaviour of the animals continuously during the journey (see figure 2.9). Video recordings were made as a back-up system in case other equipment malfunctioned.

***Figure 2.9*** *Video camera positioned in the upper corner of four of the five compartments per vehicle. Recording devices were placed in the front of the vehicle to monitor whether or not recording was activated*

## 2.4 Statistical Analysis

In order to analyse how heifers behave during transport the following questions had to be addressed by statistical analysis (see figure 2.10):

1) When and for how long do heifers lay down during the 9-hour overnight stop? (green arrow)
2) When and for how long do heifers ruminate during the 9-hour overnight stop? (green arrow)
3) Do laying and rumination activities differ between treatment groups for the first, second, third and fourth driving periods? (red arrows)
4) Do eating, ruminating and laying activity patterns differ between the treatment groups from the onset of feeding? (cyan arrow)

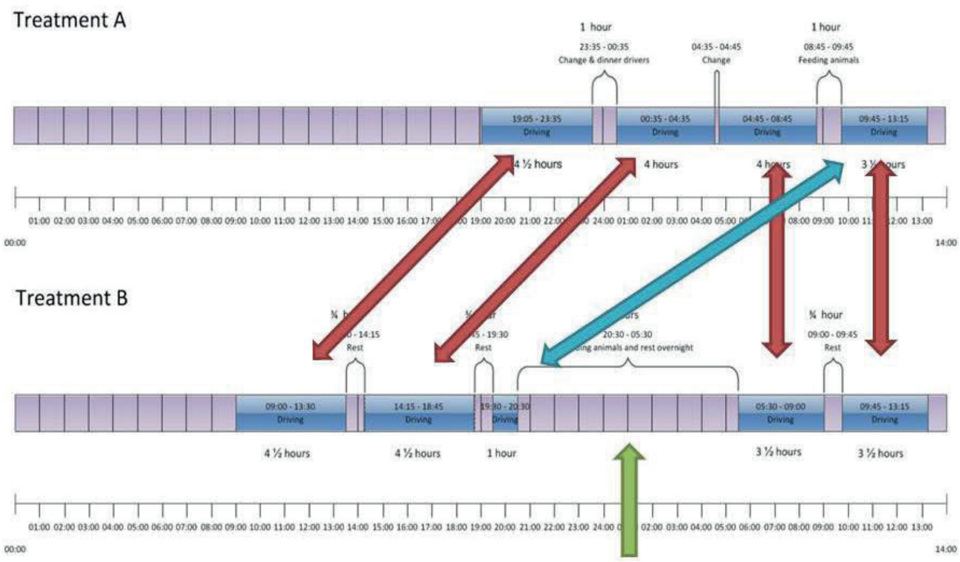

***Figure 2.10*** *Schematic presentation of the questions addressed in the statistical analysis*

For the current study it would have been of considerable importance to measure the welfare indicators before, during and after the journey. Unfortunately it appeared not possible to have any influence on the management of the heifers at the harbour in Sète. Therefore, it was not possible to do long (several hours) after transport observations. Ideally, assessment of behaviour and welfare should have continued for at least another 24 hours. Likewise, most studies assessing the effect of long transport on the health and welfare of animals need to deal with this limitation in the set-up of the study. Nevertheless, in the current study a health check was performed when heifers were unloaded, and for some transports small notes (not standardised) were taken of behaviour in the resting pens when heifers waited before loading onto the ship.

For each journey a vehicle contained 8 study heifers (2 in each study compartment). Therefore the dataset of 6 repetitions x 2 treatment journey-vehicle combinations contained 96 records with journey information at animal level. Each sensor produced repeated measurements and percentage times for lying down, ruminating and eating were recalculated on an hourly level, considered as data Y. Differences between type of vehicle and hour of the journey have been assessed by linear logistic regression analysis of the recalculated hourly data.

The Y data (have been treated as pseudo-binomial data, taking the variance to be proportional to binomial variance, i.e. $var(Y)=\sigma^2 np(1-p)$. Here p ($0<p<1$) denotes the expected relative preference Y/n of score Y. Here n stands for 100 (100%) when comparing the types of vehicle or when comparing different stages of the journey. $\sigma^2$ denotes a dispersion parameter (identified as phi in Genstat). A linear logistic model comprising fixed effects for vehicle type and stage of transportation together with random effects from transport date and study compartment within transport date have been used to describe the relationship between p and the model terms.
The fixed model reads:

$\ln\left(\frac{p}{1-p}\right) = constant$ +Tranport_type + stage (hour) of tranport + interaction.

Estimates for the dispersion parameter $\sigma^2$, main effects and F-tests for the main effects were obtained by fitting the above model using the generalized linear mixed model procedure in GenStat.

For treatment B journeys the changes in behaviour in time during the 9-hour rest period were analysed using the above model excluding the effect of Transport_type.
The latency time to the first occurrence of different behaviours (chewing, lying, ruminating) after the onset of the 9-hour rest period was analysed with the above model, after a e-log transformation of the individual latency time data. Fixed differences between study compartments were also estimated using the same model. Reverse transformation of means based on e-log scale produced median values for latency time, indicating the time-interval, after which 50% of the animals have performed this behaviour.

# 3 Results

## 3.1 Baseline recordings

Baseline recordings of eating, rumination and lying/standing behaviour were taken at the stable 24 hours (from 12 noon till 12 noon the following day) in the days prior to departure (see figure 3.1). Figures 3.2, 3.3 and 3.4 provide an illustration of these behaviours in the days before journey two. These behaviour patterns are similar for animals in treatment groups A and B.

***Figure 3.1*** *Heifers housed at the farm during the baseline recordings two days preceding the journeys*

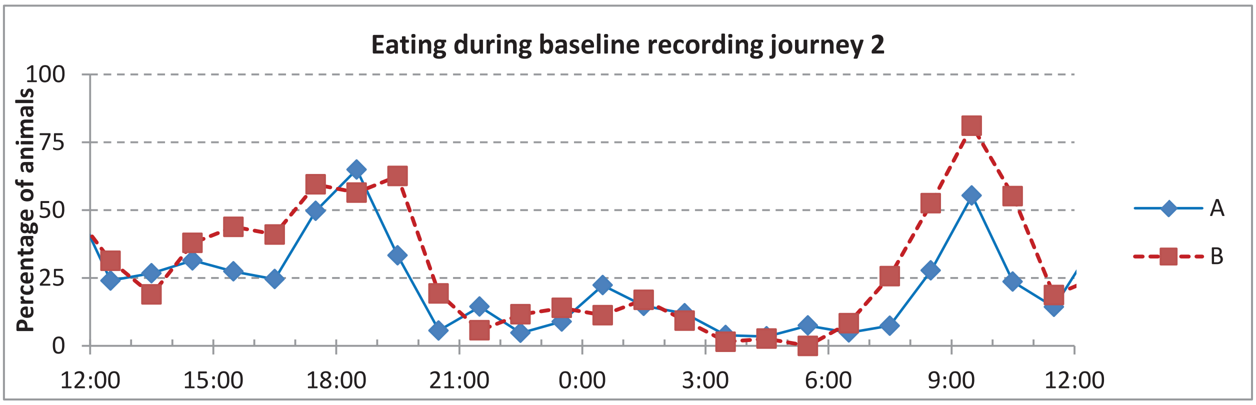


***Figure 3.2*** *Percentage of animals eating per treatment A (n=8) and B (n=8) recorded as a 24 hour baseline prior to departure*

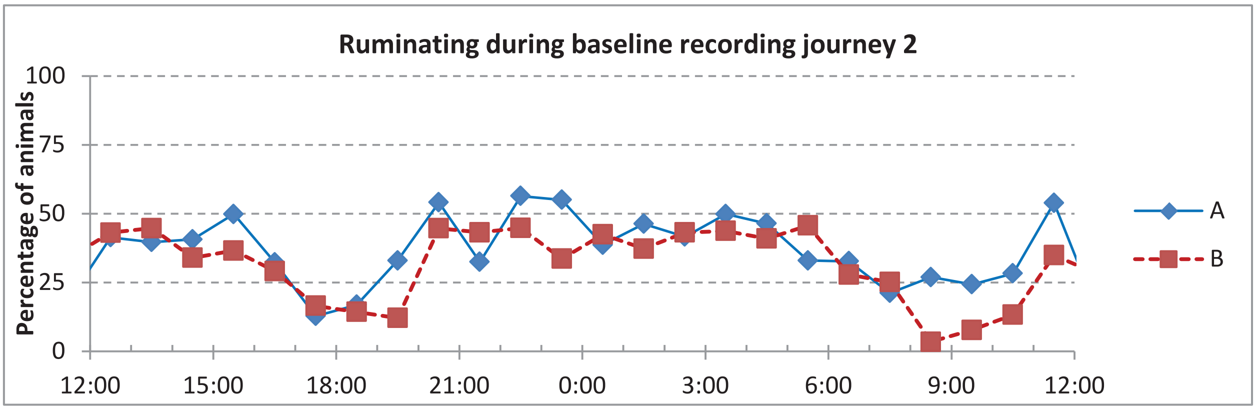


***Figure 3.3*** *Percentage of animals ruminating per treatment A (n=8) and B (n=8) recorded as a 24 hour baseline prior to departure*

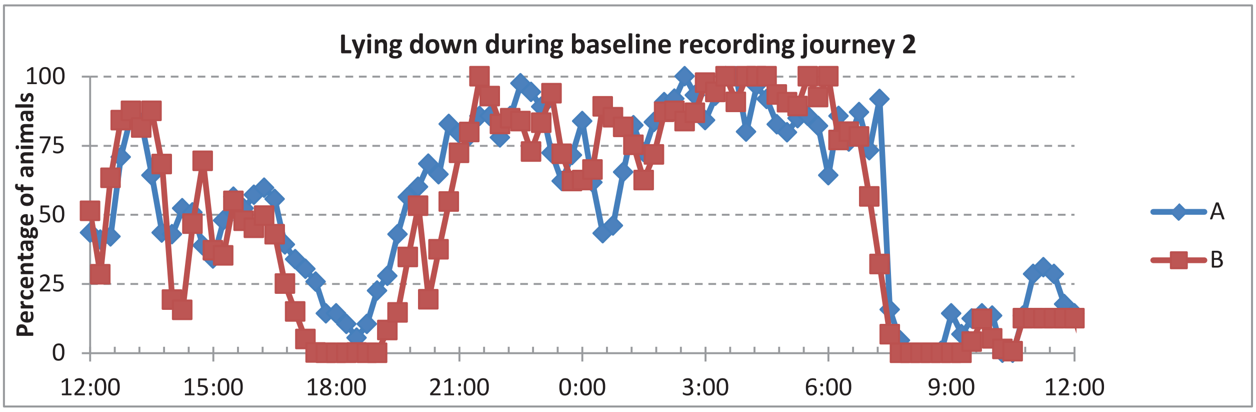


***Figure 3.4*** *Percentage of animals lying down per treatment A (n=8) and B (n=8) recorded as a 24 hour baseline prior to departure*

## 3.2 Transport data

The journey, average distance 1171 ± 21 km, took the treatment A vehicle on average 16 hours 56 minutes (± 32 minutes) to complete and the treatment B vehicle on average 27 hours and 30 minutes (± 51 minutes).

Loading the 35 heifers at the place of departure (see figure 3.5) took on average 19 minutes (± 8 minutes) and unloading at the destination (see figure 3.6 and 3.7) took on average 21 minutes (± 14 minutes).

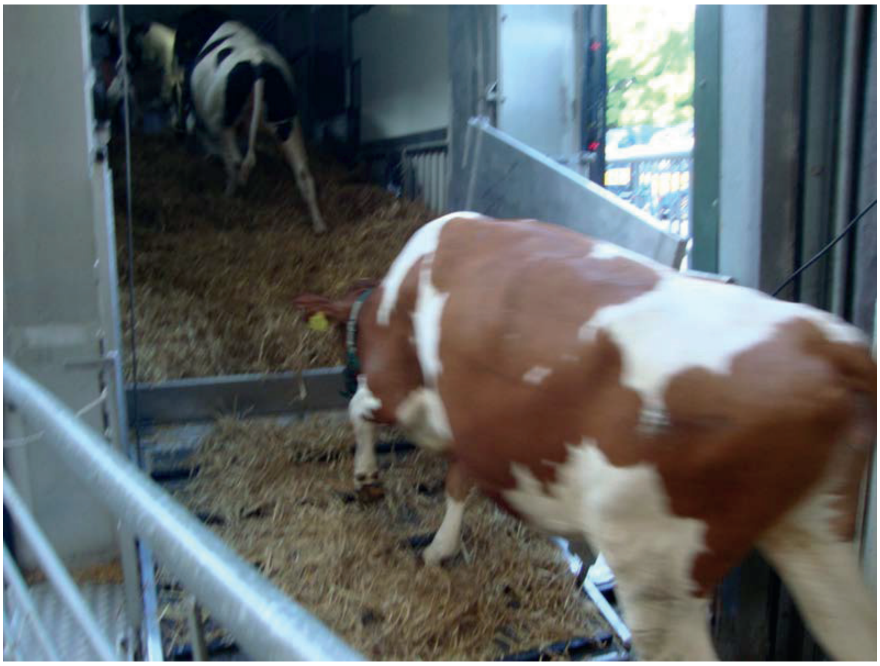

***Figure 3.5*** *Loading the heifers at the farm in Woerden (the Netherlands)*


***Figure 3.6*** *Arrival in Sète (France)*

***Figure 3.7*** *Unloading the heifers upon arrival in Sète (France)*

***Figure 3.8*** *Loading the heifers on the vessel shortly after arrival in Sète (France).This phase of the journey was not included in the current study*

## 3.3 Heart rate

During the first journey, the study animals (8 per vehicle, 2 per compartment) were equipped with data loggers to register ECG. Unfortunately, most of the ECG recordings were of insufficient quality to be accepted for further data analysis. Figure 3.9 and 3.10 show typical examples of an ECG recording of one individual with acceptable and non-acceptable recordings.

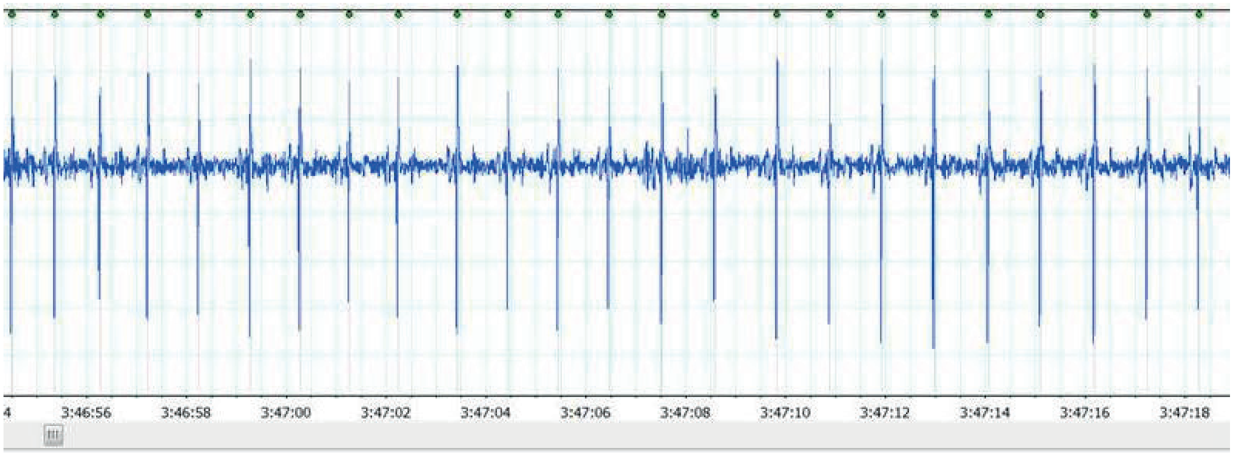


***Figure 3.9*** *Example of an acceptable (for further analysis) ECG recording of a heifer during long distance transport*

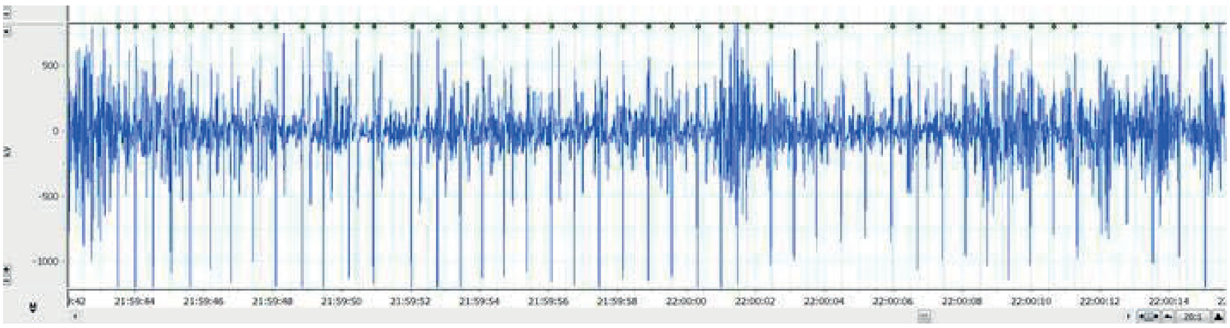

***Figure 3.10*** *Example of an unacceptable (for further analysis) ECG recording of a heifer during long distance transport*

## 3.4 Behaviour during 9-hour overnight stop

In treatment B, heifers were fed on the vehicle in the evening (between 21:00-22:00 hours) and rested for a total of 9 hours. During this 9 hour resting period, lying down and ruminating were registered continuously. Figure 3.11 shows the percentage of animals at a given time (consecutive hours from the start of the resting period) that is lying down and ruminating. Obviously, when animals were fed only very few animals were  lying down and/or ruminating. These numbers increased during the resting period and reached a maximum at six and seven hours after the start of the resting period for ruminating and lying down, respectively. A maximum percentage of 55% animals ruminating was reached, which is equivalent to 4.4 of the 8 study animals ruminating at once. For lying down, the maximum percentage was 35% which is equivalent to 2.8 study animals.

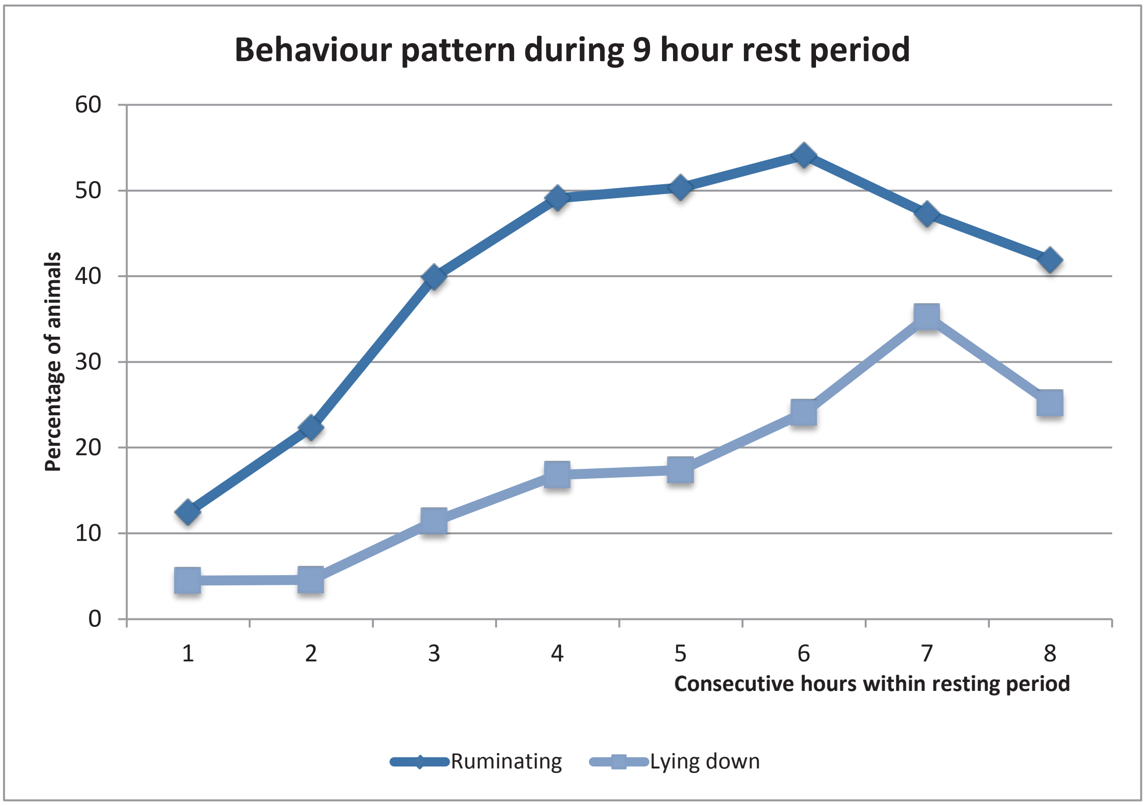


***Figure 3.11*** *Percentage of animals ruminating or lying down each hour of the 9 hour resting period. The percentage of animals is calculated as the average time that animals displayed a particular behaviour within the time period.* $\sigma^2$ *(lying down) = 41.32,* $\sigma^2$ *(ruminating) = 14.88*

Furthermore, the median time lapse between start of period and onset of rumination or lying down was analysed per compartment (see figure 3.12). Interestingly, the time lapse to onset of rumination and lying down was longest in compartment 1. However, time lapse to onset of rumination did not differ significantly between compartments; a trend towards differences between compartments in time lapse to lying down was observed ($F=2.24$, $p<0.1$).
Median time lapse per compartment to rumination was between 43 (compartment 2) and 113 (compartment 1) minutes after onset of the 9 hour resting period. Indicating that after 43 minutes 50% of the heifers in compartment 2 had started ruminating. Similarly, after 113 minutes 50% of the heifers in compartment 1 had started ruminating. All 48 heifers (Treatment B) started to ruminate before the end of the 9 hour resting period.
Four of the 48 heifers did not lie down during the full 9-hour resting period. Excluding these four heifers, the median time lapse for lying down per compartment was between 130 minutes (compartment 4) and 282 minutes (compartment 1).

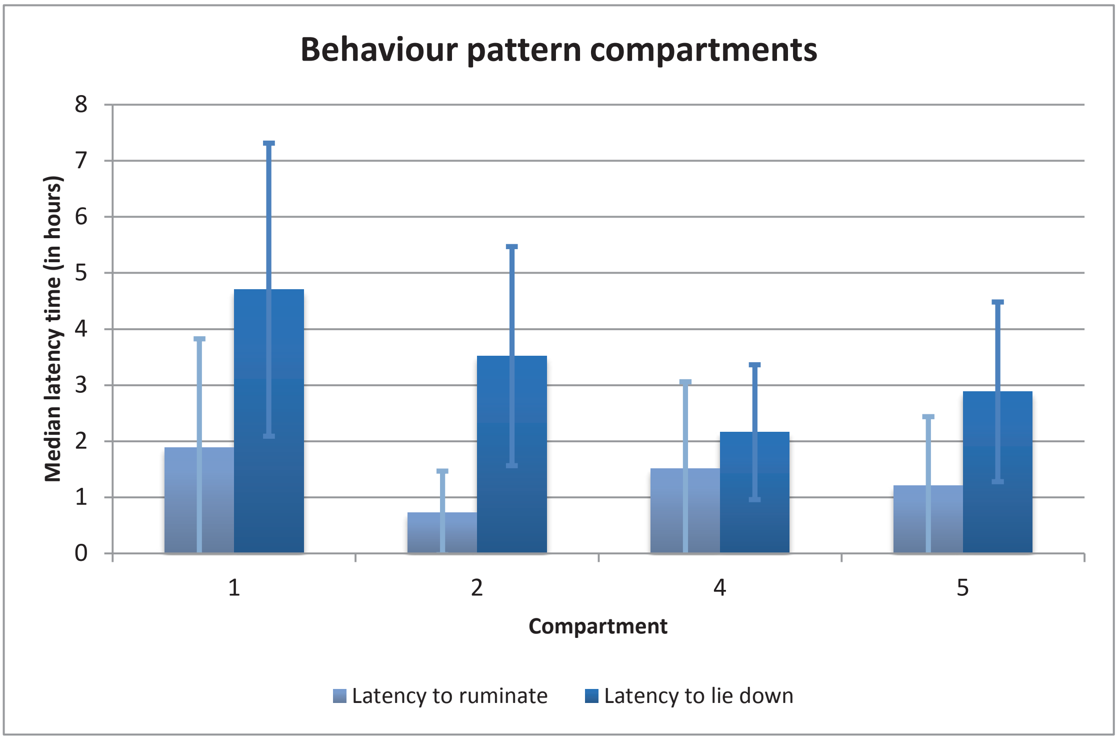


***Figure 3.12*** *Median time lapse (± stdev) per compartment to onset rumination and lying down during 9 hour resting period. Median time lapse should be interpreted as time taken for 50% of the animals to display a specific behaviour*

## 3.5 Behaviour during the four driving blocks

A complete journey consisted of four main driving blocks, all lasting for 3-4½ hours (see figure 2.10). In practice, the last driving block especially appeared to be considerably shorter: between 2-3 hours.
For treatment A there was no long resting period planned between driving blocks 2 and 3, whereas the heifers transported according to treatment B were allowed a resting period of 9 hours on the vehicle.
Another difference was that driving blocks 1 and 2 occurred at different times of the day: on treatment A this occurred in the evening and during the night, on treatment B this occurred during the day.
In the following paragraphs the same driving blocks are compared between treatment A and treatment B.

### 3.5.1 Lying down

In the first driving block of 4½ hours, hardly any heifers were lying down. This percentage increased during subsequent driving blocks (see figure 3.13). In the fourth driving block there were significantly ($F=7.52$, $p<0.05$) more animals lying down in treatment B than in treatment A. In the last 2-3 hours of the journey (driving block 4), 30% of the heifers allowed a resting period of 9 hours overnight were lying down, whereas this was 11% for the heifers that were not allowed a 9-hour resting period on the vehicle.

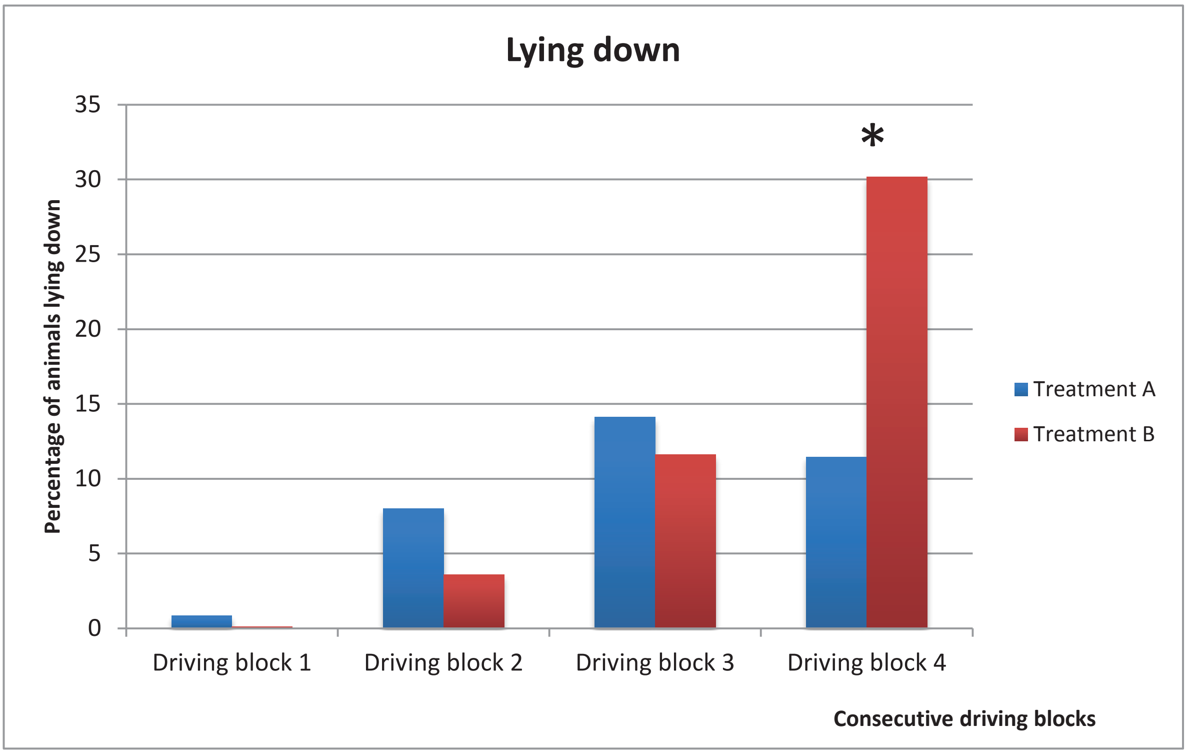


***Figure 3.13*** *Percentage of animals that is lying down during the 4 consecutive driving blocks. The percentage of animals is calculated as the average time that all animals displayed a specific behaviour in each period*

More detailed analysis within each driving block, revealed a significant increase and decrease in lying down during the 4 hours in driving block 2 (figure 3.14; $F=6.66$, $p<0.05$). The highest percentage of animals lying down was after 3 hours into the second driving block. Although not significant, heifers in treatment A attributed most to this result.

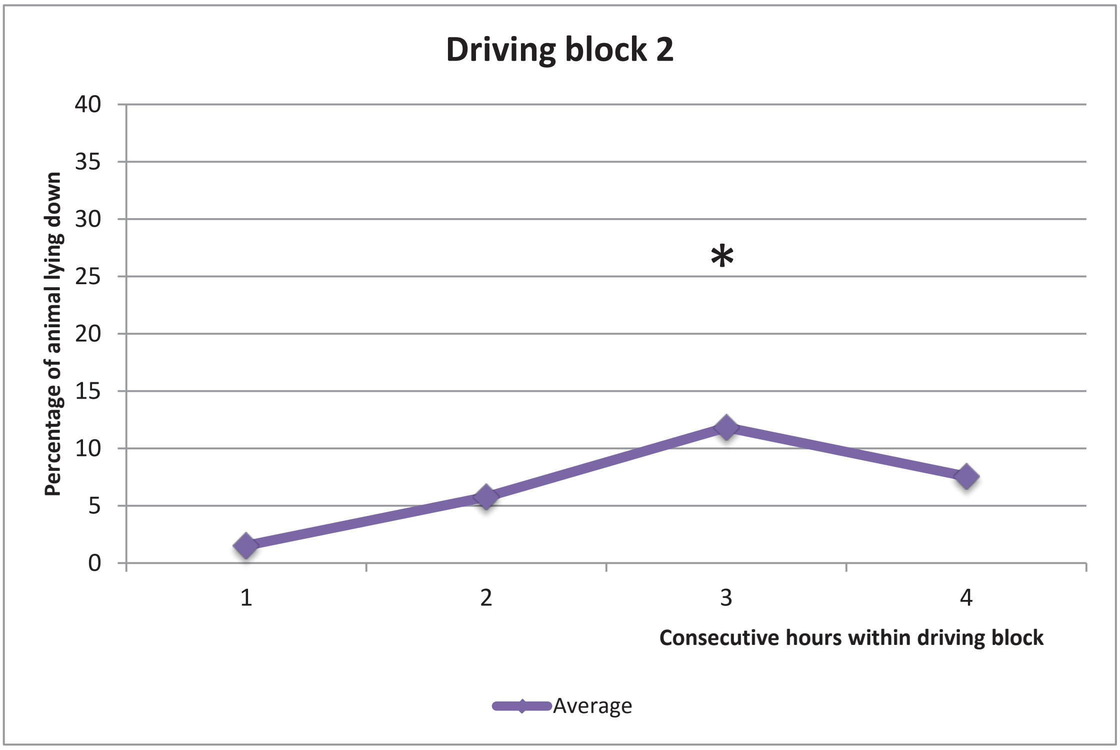


***Figure 3.14*** *Percentage of animals lying down during the 4 consecutive hours in driving block 2. Percentages are averaged over the two treatment groups. The percentage of animals is calculated as the average time all animals displayed a specific behaviour within a specific period.* $\sigma^2$ *(lying down) = 16.27*

### 3.5.2 Ruminating

The percentage of animals ruminating increased as the journey progressed (see figure 3.15), with a peak during the second driving block for the animals in treatment A (19% of animals ruminating) and the highest percentage of animals ruminating in block 4 for treatment B (25 % of animals ruminating). Differences between treatments were not significant within driving blocks.

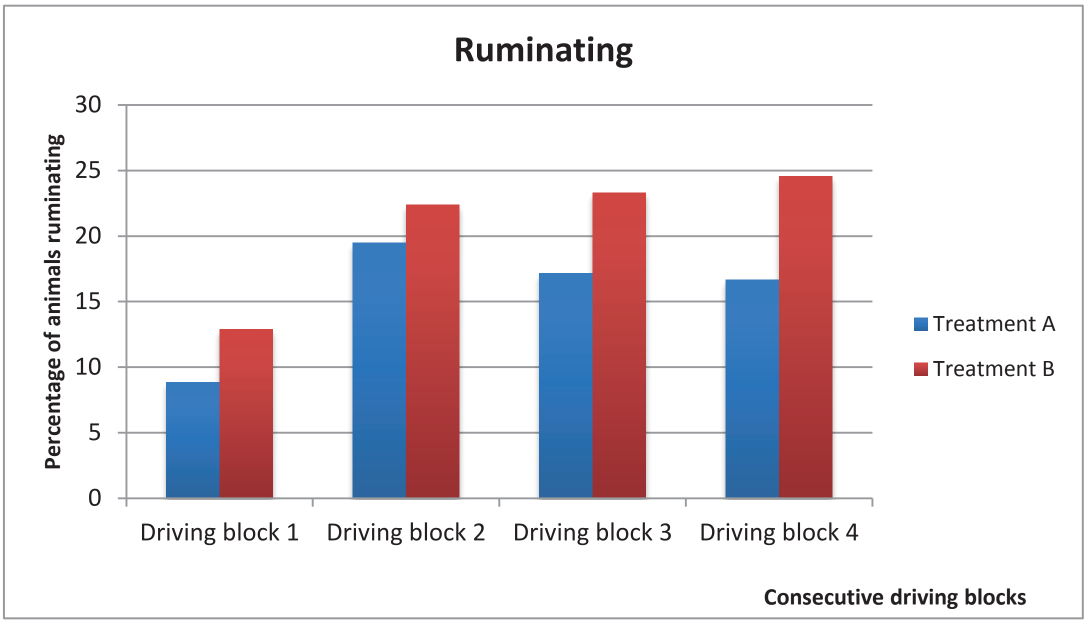

***Figure 3.15*** *Percentage of animals on both treatments ruminating during the four consecutive driving blocks. The percentage of animals is calculated as the average time that all animals display a specific behaviour during a specific period*

Furthermore, it was found that the heifers in treatment B showed a significant increase in percentage of animals ruminating within the 4½ hours of block 1 (figure 3.16; F=2.73, p<0.05).

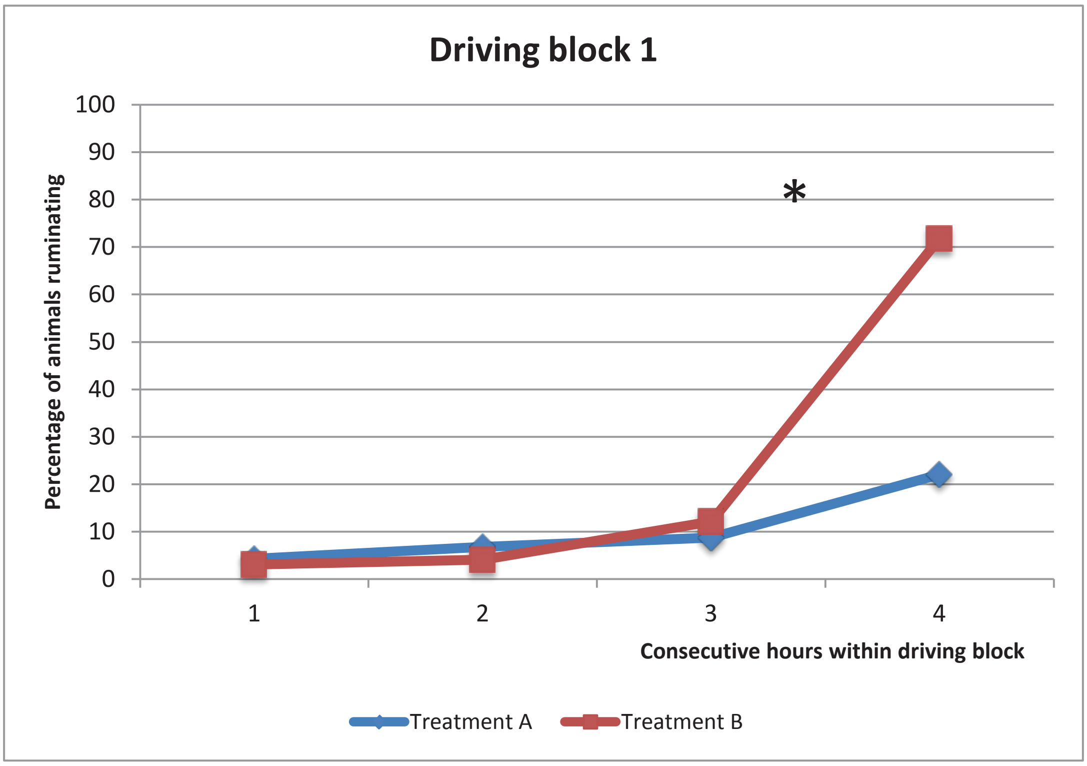


***Figure 3.16*** *Percentage of animals ruminating during the 4 consecutive hours within driving block 1. The percentage of animals is calculated as the average time of all animals displaying a specific behaviour within a specific period. σ2 (ruminating) = 17.03*

## 3.6 Behaviour from onset feeding

Heifers on both treatment groups were fed during the journey. Animals in treatment group A were fed between driving blocks 3 and 4, treatment group B animals were fed after driving block 2. The following paragraphs, present the results of the first two hours after feeding commenced.

### 3.6.1 Lying down

In the first hour after onset of feeding only a very small percentage (1-4%) of animals were lying down. This percentage increased within the second hour after onset feeding. The increase differed significantly between treatment groups (figure 3.17; F=6.59, p<0.05). Heifers in the treatment B vehicle that had stopped for 9 hours had a lower starting percentage of animals lying down (0.5%) than the other treatment group (4%). The percentage of animals lying down in each treatment group increased to around 5% during the second hour after onset of feeding.

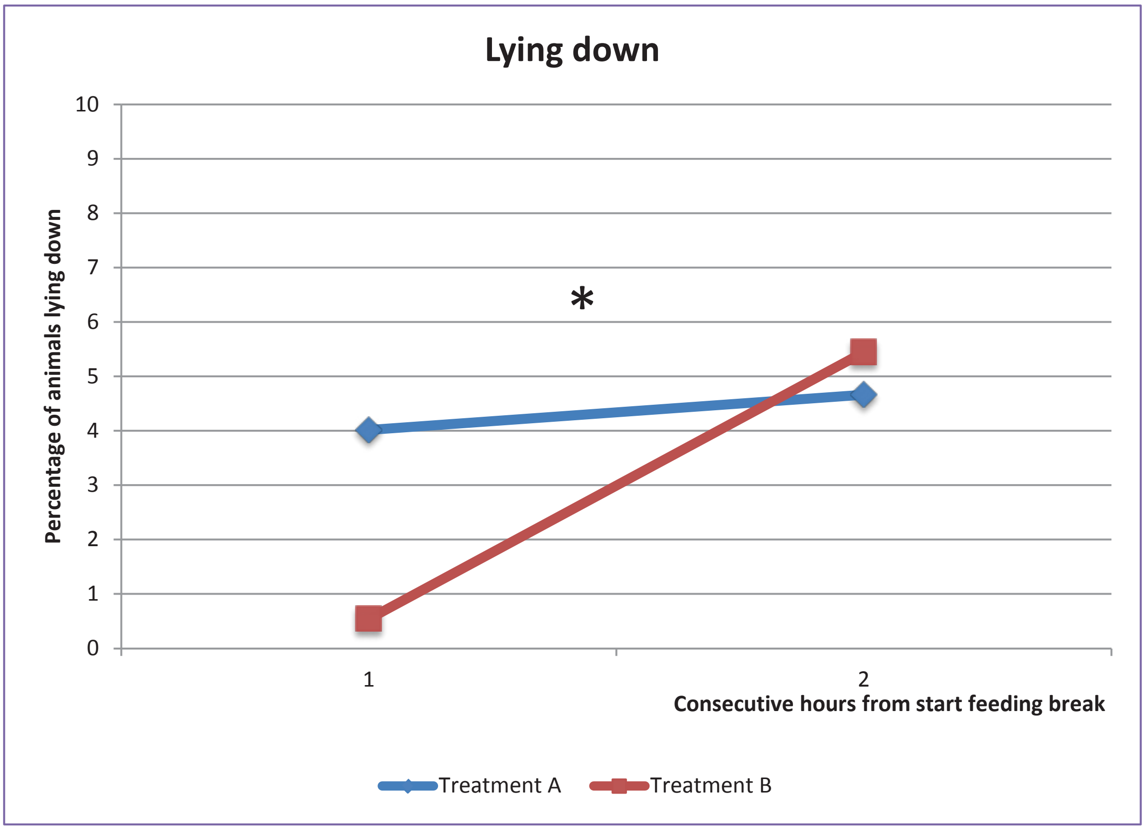


***Figure 3.17*** *Percentage of animals lying down during the two hours following onset of feeding. The percentage of animals is calculated as the average time of all animals displaying a specific behaviour within a specific period.* $\sigma^2$ *(lying down) = 15.02*

### 3.6.2 Eating

The percentage of animals eating after the start of the feeding period, differed significantly between treatment groups (figure 3.18; F=20.56, p<0.001). The percentage of heifers eating that were allowed a 9-hour resting period was 52% in the first two hours following the onset of feeding, this was 19% in the other group (treatment A). More detailed analysis revealed that the percentage of animals eating decreased between the first and second hour after onset of feeding. This decrease differed significantly between the two treatment groups (figure 3.19; F=4.92, p<0.05). Heifers in treatment group A displayed a significantly steeper decrease in eating activity that those in treatment group B.

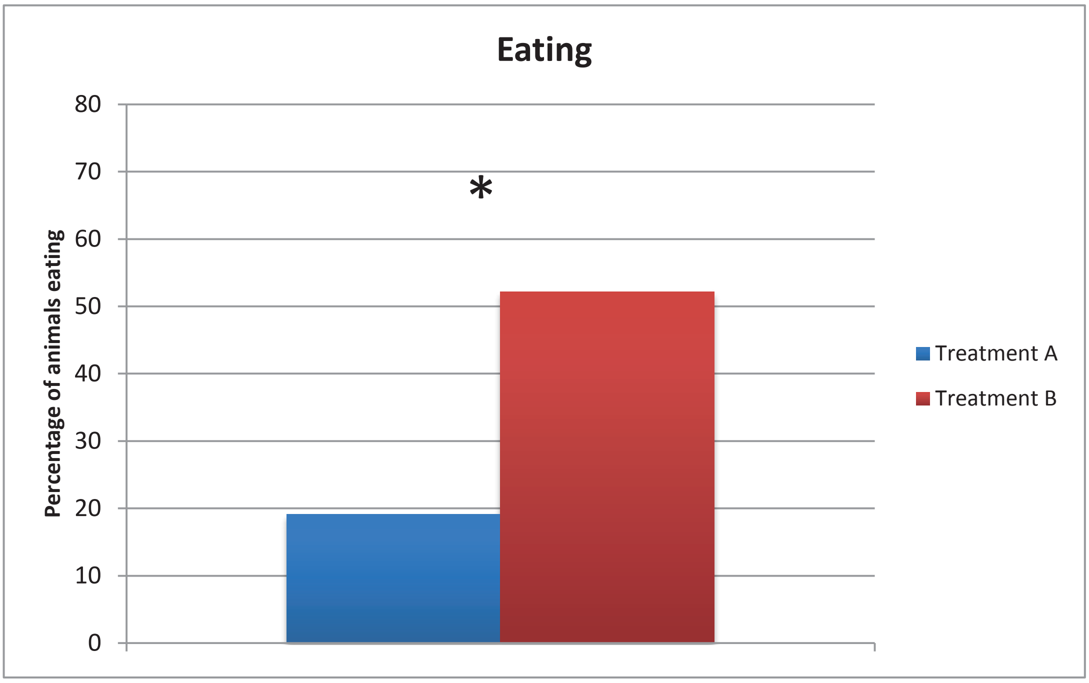


***Figure 3.18*** *Percentage of animals eating during the two hours following onset of feeding. Averages over the two hour period. The percentage of animals is calculated as the average time that all animals displayed a specific behaviour during a specific period*

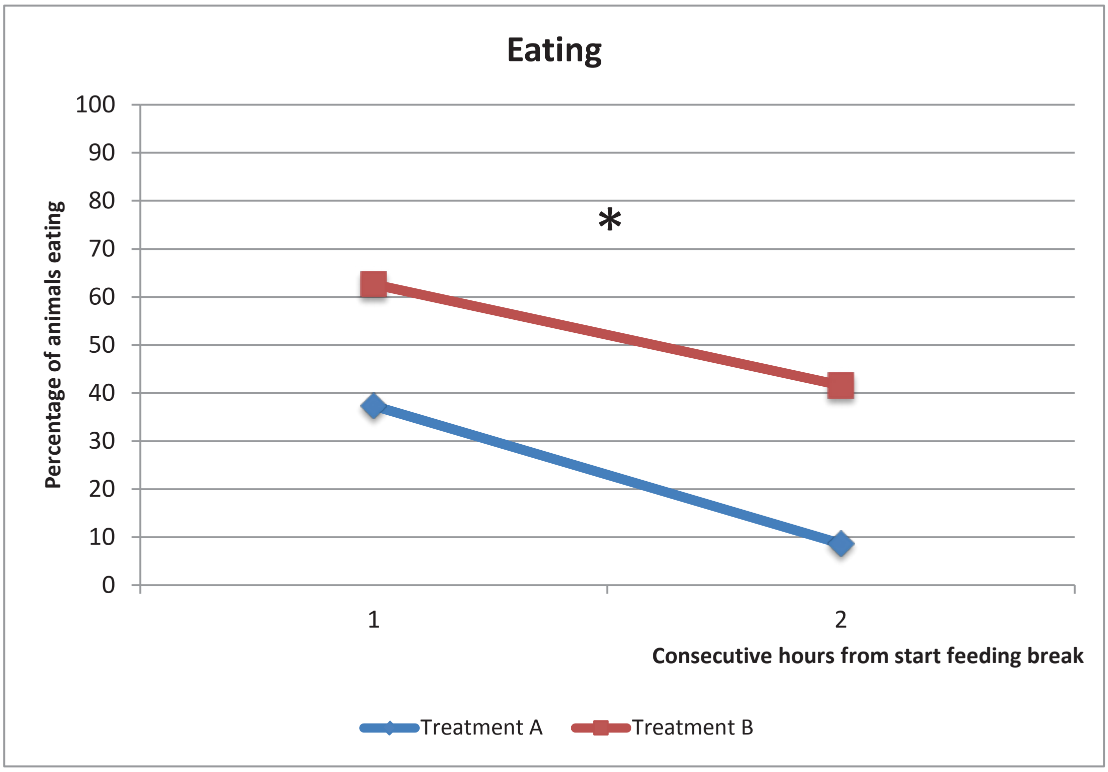


***Figure 3.19*** *Percentage of animals eating during the two hours following onset of feeding. Percentages for the first and second hour, for both treatments. The percentage of animals is calculated as the average time that all animals displayed a specific behaviour within a specific period.* $\sigma^2$ *(eating) = 16.18*

### 3.6.3 Ruminating

In addition to the percentage of animals eating, it was also calculated that there was a significant difference in the increase of animals ruminating between treatment groups after the onset of feeding. As can be seen in figure 3.20, the increase in animals ruminating was significantly more profound in treatment group B ($F=5.57$, $p<0.05$).

Within the first hour following the onset of feeding approximately 15% of the animals had started ruminating this rose to 28% during the second hour in treatment group B (9 h resting period) and to 17% in group A.

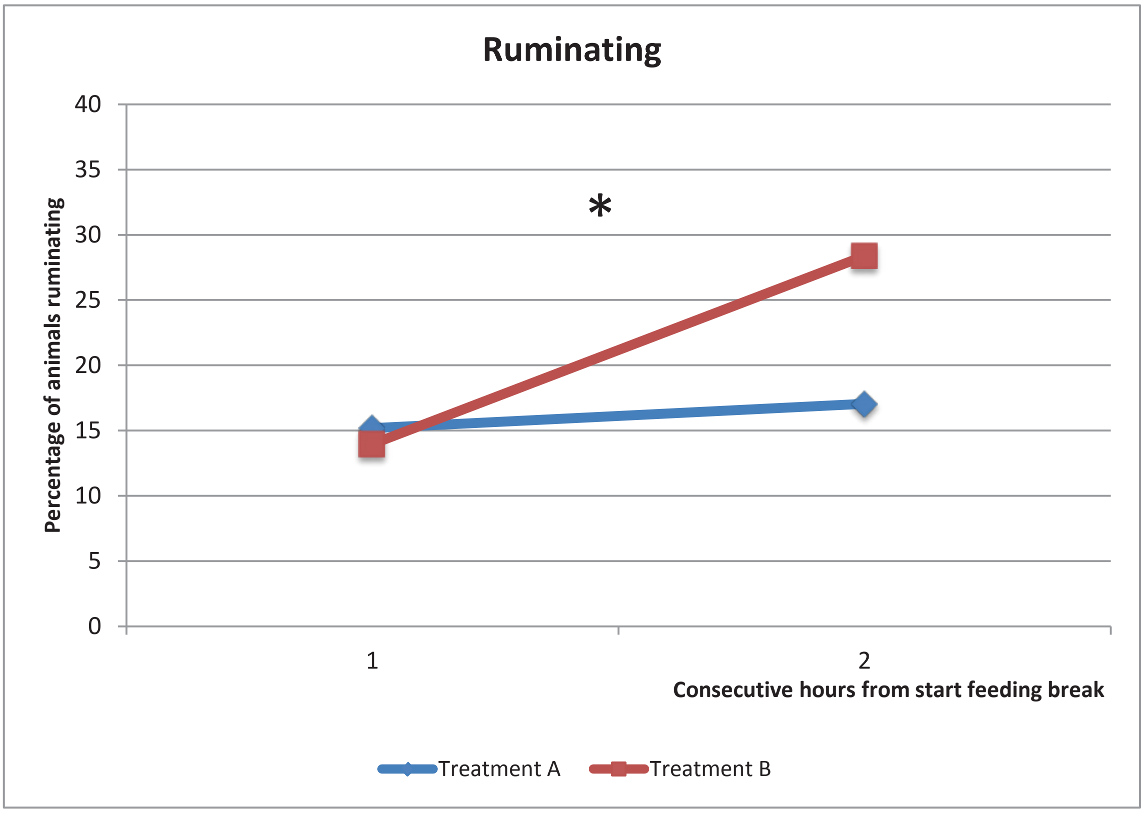


***Figure 3.20*** *Percentage of ruminating animals during the two hours following onset of feeding. Percentages for the first and second hour, for both treatments. The percentage of animals is calculated as the average time that all animals displayed a specific behaviour within a specific period.* $\sigma^2$ *(ruminating) = 16.35*

## 3.7 Water consumption

The water usage was determined for 5 of the 12 journeys (3 treatment A; 2 treatment B) based on weight difference data received from the transport company. Based on calculations, average heifers used 8.8 ± 6.7 litre per individual throughout the journey. Heifers transported in the treatment A vehicle used on average 4.8 ± 4.6 litre per individual, whereas those transported in the treatment B vehicle used on average 5.4 ± 8.8 litre per individual (see also Appendix 1). It was not further analysed why there was on average only 0.6 litre usage per animal difference for an extra 9 hours of transport.

## 3.8 Compartment temperature

Kleventa® temperature sensors were located in all 5 compartments and. Additionally, a second ATV-01 sensor was placed in the four study compartments as back-up registration for compartment temperature. Although not statistically analysed, both systems show similar patterns and values (see figure 3.21).

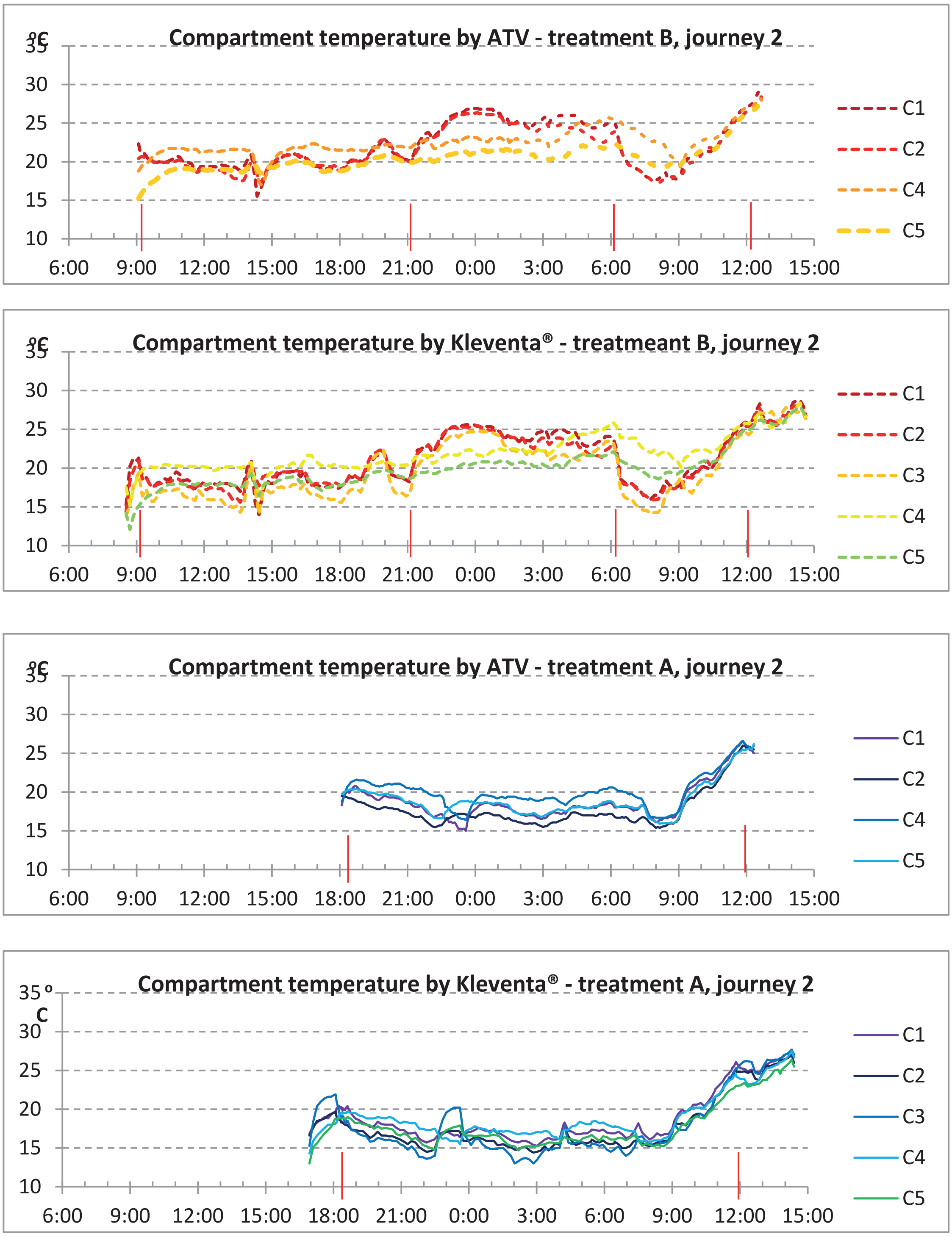


***Figure 3.21*** *Compartment temperatures in treatment A and treatment B vehicles, on the second journey. Temperature measured with ATV-01 data loggers and with Kleventa® sensor system. Vertical red lines indicate onset and end of driving periods*

As illustrated in figure 3.21 (journey 2) treatment A compartment temperature remained between 15 and 20° Celsius during most of the evening and night. In the morning, as outside temperature increases, compartment temperature also rises to approximately 25 degrees.
For treatment B, the heifers were also transported during the daytime and compartment temperature remains between 15 and 20 degrees but for one compartment (C4) increases above 20 degrees Celsius. From the moment the vehicle stops for the feeding and 9 hour break, compartment temperature increases to 20-25 degrees Celsius and for C1 and C2 rises above 25 degrees Celsius. In the morning after 06:00, compartment temperatures drop initially during driving and return to values similar to those for treatment A at time of arrival.
Average compartment temperatures for all journeys and treatments are given in Appendix 1.

## 3.9 Driving speed

Driving speed was registered using the Kleventa® system throughout all 6 journeys for both treatment groups. Figure 3.22 provides an illustration of the driving speeds of the treatment A and B vehicles. As shown, treatment B had a 9-hour overnight stop, that commenced around 21:00 hours, the journey was resumed around 06:00 next morning.

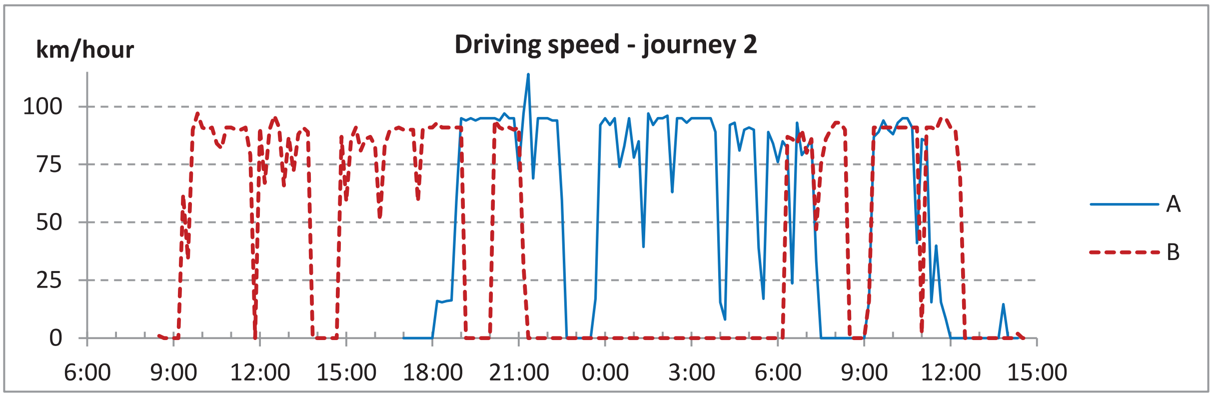


***Figure 3.22*** *Driving speeds of treatment A and treatment B vehicles, (second journey) registered using the Kleventa® system*

## 3.10 Clinical condition

Results from the clinical condition assessment are presented in table 3.1. Clinical assessment was performed before the animals were equipped, prior to loading on day of departure, and immediately after unloading upon arrival at the harbour.
As shown in table 3.1 all animals were in good condition prior to transport and were assessed to be in similar condition after unloading. However, small differences were observed for certain parameters between treatment groups after unloading (i.e. respiration rate, ear temperature, behaviour). These differences were not considered to be consistent with treatment effects.
In general, the animals were considered to be more tense after the journey than before departure. This was not considered to differ per treatment.

**Table 3.1**
*Results (% study animals) of the clinical assessment prior to baseline recording, before loading, after unloading.*

| | | Baseline recording | Before loading | After unloading | |
|---|---|---|---|---|---|
| | | | | ***Treatment A*** | ***Treatment B*** |
| **Respiration rate** | fast | 0.0% | 0.0% | 14.9% | 19.2% |
| | slow | 100.0% | 100.0% | 85.1% | 80.7% |
| **Respiration rate** | flat | 0.0% | 0.0% | 0.0% | 0.0% |
| | deep | 100.0% | 100.0% | 100.0% | 100.0% |
| **Respiration sounds** | yes | 0.0% | 0.0% | 0.0% | 0.0% |
| | no | 100.0% | 100.0% | 100.0% | 100.0% |
| **Short of breath** | yes | 0.0% | 0.0% | 0.0% | 0.0% |
| | no | 100.0% | 100.0% | 100.0% | 100.0% |
| **Rectal temp** | | 38.6 | 38.5 | 38.7 | 38.7 |
| **Ears temperature** | warm | 80.2% | 89.1% | 77.8% | 93.8% |
| | normal | 0.0% | 2.2% | 20.0% | 6.3% |
| | cold | 19.8% | 8.7% | 2.2% | 0.0% |
| **Coat** | wet | 2.1% | 1.0% | 0.0% | 4.3% |
| | dry | 97.9% | 99.0% | 100.0% | 95.7% |
| **Cleanliness** | dirty | 4.2% | 10.4% | 4.2% | 6.4% |
| | clean | 95.8% | 89.6% | 95.8% | 93.6% |
| **Normal claws** | yes | 100.0% | 100.0% | 100.0% | 100.0% |
| | no | 0.0% | 0.0% | 0.0% | 0.0% |
| **Mucous membranes** | normal | 82.1% | 92.5% | 91.7% | 95.7% |
| | dark | 0.0% | 0.0% | 0.0% | 0.0% |
| | pale | 17.9% | 7.5% | 8.3% | 4.3% |
| **Behaviour** | normal | 39.1% | 45.8% | 39.1% | 36.2% |
| | tense | 23.9% | 17.7% | 45.7% | 55.3% |
| | quiet | 37.0% | 36.5% | 15.2% | 8.5% |
| | aggressive | 0.0% | 0.0% | 0.0% | 0.0% |
| **Normal posture** | yes | 100.0% | 100.0% | 100.0% | 100.0% |
| | no | 0.0% | 0.0% | 0.0% | 0.0% |
| **Locomotion** | lame | 0.0% | 0.0% | 0.0% | 0.0% |
| | sound | 31.2% | 92.7% | 100.0% | 100.0% |
| | not visible | 68.8% | 7.3% | 0.0% | 0.0% |
| **Dung** | normal | 51.0% | 19.8% | 4.4% | 8.5% |
| | thick | 2.1% | 3.1% | 2.2% | 2.1% |
| | thin | 3.1% | 3.1% | 0.0% | 2.1% |
| | not visible | 43.8% | 74.0% | 93.4% | 87.3% |
| **Hindquarters** | clean | 98.9% | 90.6% | 97.9% | 97.9% |
| | dirty | 1.1% | 9.4% | 2.1% | 2.1% |
| **Tail** | clean | 98.9% | 94.8% | 100.0% | 100.0% |
| | dirty | 1.1% | 5.2% | 0.0% | 0.0% |
| **Small scratches** | yes | 19.8% | 32.3% | 33.3% | 33.3% |
| | no | 80.2% | 67.7% | 66.7% | 66.7% |

# 4 Discussion and conclusions

## 4.1 Methodological constraints of the study

The current study is intended as a step towards formulating an hypothesis on long distance road transportation. Implying that the performance constraints on this type of study in a commercial setting and the limitations of standardisation dictate that the results should be interpreted with care. Nevertheless, significant results yielded in this study are true for the journey and the heifers that were being assessed at that time. These results may be used to hypothesize for future studies and practices.

The constraints relevant to this study that complicated standardisation were:

- a limited number of journeys that could be assessed (financial and time constraint)
- limited possibilities for the drivers to follow the agreed driving and resting schedules exactly (practical constraint)
- inability to perform standardised measurements for a longer period after completion of the journey (practical constraint)
- confounding factors that could have affected the results; e.g. time of the day, weather, season, driving conditions, driving skills etc.

## 4.2 Interpretation of the results

### 4.2.1 Heart rate measurements

Analysis of ECG data from the first journey was challenging since only 3 (out of 16) animals provided acceptable heart rate traces for the whole journey. All other animals provided incomplete or corrupt ECG recordings. Some ECG electrodes lost contact during the transport, others displayed many artefacts. Attempts to filter the artefacts, was found to be very time-consuming.

Based on experiences from earlier studies (Lambooij et al., 2012) where heart rate in cattle increased during the handling phases (loading and unloading) and retained normal values during transport no extreme heart rate values were expected within the few ECG recordings analysed form the first journey. Therefore, it was jointly decided to abandon heart rate registration for the remaining 5 journeys.

### 4.2.2 Behaviour of heifers in the vehicle during the 9-hour overnight break

All heifers were fed at the beginning of the 9-hour overnight break. This was at approximately 21:00 hours. As shown in figure 3.11 the percentage of animals lying down and ruminating was low during the first two hours following the onset of feeding but increased as time progressed. As shown in figure 3.19, more than 60% of the heifers in this vehicle (treatment B), were eating in the first hour after feeding. The percentage of animals that began to ruminate increased rapidly during the next few hours and reached a maximum of 55% 6 hours after the beginning of the 9-hour resting period. Diurnal rumination patterns have been described for heifers (Jaster and Murphy, 1983). The pattern of feeding, eating and ruminating in the present study follows the same pattern as described in studies by Schirmann et al. (2012). In those studies dairy cattle spent most of the time ruminating at night. When fed at 16:00 hours, they displayed an eating peak between 17:00 – 19:00 hours and a rumination peak between 22:00 and 05:00 hours (Schirmann et al., 2012). The baseline recordings (on farm) of the heifers of this current study (see paragraph 3.1), illustrate that the animals started eating around 18:00 hours, and maximum percentages of ruminating animals fall between 21:00 and

06:00 hours. So it is hypothesised that the time between onset feeding and peak ruminating on farm lies between 3-5 hours. The current study indicates that the same eating-ruminating interval is preserved for heifers fed and rested on the vehicle, even if they are fed at a later time of the day (21:00 instead of 16:00-18:00 hours). The average percentage of animals ruminating during the peak rumination hour was 55%. Compared to the example of the baseline recordings from the second journey (figure 3.3) this percentage was higher in comparison to the highest average percentages of ruminating animals during baseline recording: 45-55%. Summarizing it appeared that rumination during the 9-hour break was not affected.

Lying behaviour follows a diurnal pattern, similar to ruminating. Several studies have investigated the total daily lying times. For example, pregnant cattle were found to have 6.8 lying bouts or 10.5 hours of lying down each day. Cattle in late gestation were found to have 10 bouts and 11.6 hours lying down per day (Tolkamp et al., 2010). Jensen et al. (2005) found that approximately 3-month pregnant heifers have a strong demand for 12 to 13 hours of lying down every 24 hours. In the study of Tolkamp et al. (2010) between 60-90% of the non-pregnant dairy cattle was lying down during the night and 20-50% during daytime hours.

The total lying time per heifer in the present study was not analysed. From figure 3.11 it is shown that 35% of the heifers would lie down at the same time 7 hours after the onset of the 9-hour overnight break. This percentage of animals lying down simultaneously on the vehicle is considerably lower compared to the percentage of animals lying down simultaneously on farm or in the study of Tolkamp et al. (2010). In figure 3.4 it is illustrated that between 75-100% of the animals was lying down during the night; which is similar to other studies.

Remarkably, 4 of the 48 treatment B study heifers did not lie down at all during the 9-hour overnight stop. Apparently, the first of these 4 events occurred during the 4$^{th}$ journey, the remaining three occurred during the 6$^{th}$ journey. Heifers that did not lie down were transported in compartment 1 (2 heifers, different journeys), compartment 2 and compartment 4. Continual standing could have been caused by unfamiliarity with the situation (animals were not feeling sufficiently ‘safe’ to lie down) and/or by lack of space (although compartment sizes were according to EC Regulation 1/2005 heifers could have been using the space not as efficient as possible) (see figure 4.1). It needs to be stressed that heifers’ behaviour within compartments was not independent; meaning, that if one animal was more active than the others this inevitable decreased the chance of other animals going to lie down. In summary, the lying behaviour of the heifers  during the 9-hour stop in the present study was not the same as on farm.

***Figure 4.1*** *Heifers lying down and standing in one of the compartments upon arrival in Sète*

Remarkably, the time lapse between onset of the 9-hour resting period and lying down or rumination was longest in compartment 1. Compartment 1 was situated on the upper deck, directly behind the driver's cabin. It is known that driving style and suspension of the vehicle can effect lying behaviour. In a study with sheep it was found that driving events were also responsible for many interruptions to both lying behaviour and rumination. Clear benefits observed for driving on the motorway compared to driving on a single carriageway. Fewer incidences of loss of balance, more lying down, more rumination and fewer disturbances amongst sheep were found during motorway driving (Cockram et al., 2004). It is anticipated that any driving event has a greater effect on heifer behaviour on the upper deck compared to heifers travelling on the lower deck. Although the larger lapse times (to lie down and rumination) were applicable to the 9-hour resting period, prior experience of the heifers during the two driving blocks preceding the resting period may have influenced this behaviour. Further study is needed to examine more closely why the heifers in compartment 1waited more than 4 hours before lying down.

### 4.2.3 Heifer behaviour during driving

During the driving blocks, the percentage of animals ruminating remained relatively low, up to 25% (see figure 3.15), which is considerably lower than when the vehicle is standing still (figure 3.11: 55% of the animals in treatment B were ruminating at one point during the 9-hour overnight stop). No differences were observed in percentage ruminating animals between treatment groups during all four driving blocks.

Based on the practical experiences of the drivers, it was expected that most heifers would lie down within 2-3 hours after departure. However, the percentage of heifers lying down during driving in the current study was considerably lower. In the first two driving blocks (the first 10 hours of the journey), less than 10% of the heifers was lying down. Longer into the journey, the percentage of heifers lying down increased in both treatment groups, but did not exceed 30%. This implies that during driving, in one compartment with 7 heifers, 2 heifers were lying down at the same time. Extrapolating to the whole, 10-11 of the 35 heifers were lying down at the same time. Petherick and Phillips (2009) have calculated space allowances for confined livestock from allometric principles. They argued that animals need more space to move between standing and lying as compared to physically space they occupy when lying down. Based on limited evidence, they suggest that the amount of space needed to move between standing and lying is about the same amount as required for lateral recumbent lying. Space allocated according to the lateral recumbent equation is area ($m^2$) = $0.047W^{(0.66)}$, with W being the weight of the animal. For the heifers in the current study, with a mean weight of 546 kg, this would mean that they would need 3.01 $m^2$ per animal to move between lying down and standing. This is almost twice as much as the area for heifers in the current study. Hence, a small number of animals lying down at the same time is conform allometric principles.

Although not systematically recorded, the researchers noted that all animals, regardless treatment group, would lie down and ruminate soon after they were unloaded and placed in resting pens at the harbour. Despite that this has not been recorded systematically the impression was that all animals displayed rebound behaviour to compensate for lying down and rumination deficit.

As considered by SCAHAW (2002), careful driving, especially in bends and corners on the route, together with acceleration and braking, have a substantial effect on the cattle welfare during transportation. Also in a study of Peeters et al. (2008) it was shown that driving style mainly had an effect on the longitudinal and lateral accelerations. Increasing acceleration resulted in an increase in the proportion of pigs standing during the journey and a decrease in the proportion of pigs lying down. In the present study, no objective measurements were taken regarding driving style; but the drivers chosen for the current study were acknowledged to drive in a passive and careful manner. Nevertheless, based on the results from the present study it can be hypothesised that when the vehicle is moving, heifers are less prone to ruminate and to lie down.

Although the percentage of animals lying down during driving remains low in the fourth driving block, significantly more heifers lying down in treatment group B than in treatment group A. One reason for this finding could be that the heifers of group B have experienced during the 9-hour overnight stop that it is physically possible or ‘safe’ to lie down to a certain extent and hence were lying down during the latter driving blocks. Yet another reason for this significant difference could be the fact that the heifers in treatment group A were fed between driving blocks 3 and 4, while those in treatment group B were fed 10 hours earlier (after the second driving block). In treatment A the heifers only had a limited time to eat, after which the vehicle continued the journey (driving block 4).

Satisfying the motivation to lie down is important for the welfare of dairy cows (Wierenga and Hopster, 1990). Since lying down in this present study differed from expected patterns lying down events were calculated for each heifer and given in figure 4.2. For both treatment groups, several heifers did not lie down at all during the whole journey. The average absolute duration of lying down in treatment group A was 1 hour 48 minutes (±2 hours) and 3 hours and 18 minute (±2 hours and 16 minutes) for treatment B. Total duration of lying down corrected for duration of the journey revealed that in treatment group A heifers were lying down for 10.7% (± 11.9%) of the total journey time and in treatment group B 12.5% (± 8.7%). In treatment group A 7 of the 48 heifers did not lie down at all during the 16-17 hours journey; in treatment group B 1 of the 48 heifers did not lie down during the 27-28 hour journey. The reason why some animals did not lie down is still to be speculated. It needs to be stressed that whether or not animals lie down is also very dependent on the other animals in the compartment. When one of the animals is active, the chance other animals will lie down decreases considerably.

As with the heifers not lying down during the 9-hour overnight stop, it may have been a) feeling 'unsafe' and/or b) lack of space. Yet another reason can have attributed to this difference between heifers in vehicle A and B: the whole 'transport experience'. The transportation scheme of vehicle A was focussed on driving with only minor stops, the transportation scheme of vehicle B was focussed on having more and longer breaks, in which the animals could have taken some rest and/or experienced that they could lie down in the vehicle when it was standing still. This 'transport experience' may have been perceived different by the animals.

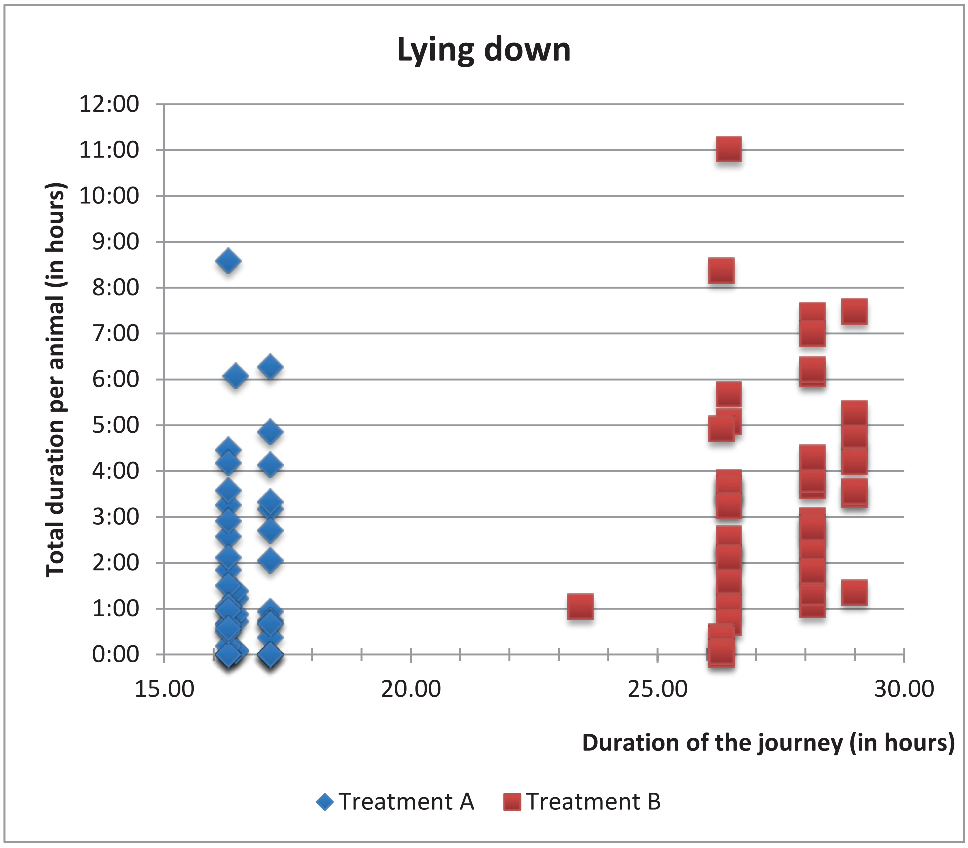


***Figure 4.2*** *Total duration of lying down for each heifer during the whole journey*

### 4.2.4 Eating, ruminating and lying down after feeding on the vehicle

One of the requirements of Regulation (EC) 1/2005 is that cattle on transport are watered, and fed if necessary, after 14 hours of travel. Feed and water deprivation can have serious consequences for ruminant health. This can disrupt the rumen ecosystem and cause microbial death resulting in the release of microbial endotoxins that elicit the bovine acute-phase response (Marques et al., 2012). Therefore, during the commercial transport that was engaged in the present study, heifers had ad libitum access to water and were fed once at a pre-set time. Despite differences as a) time of the day that the heifers were fed (clock time: 09:00 hours treatment A versus 21:00 hours treatment B), and b) the duration of the journey until feeding (14 hours treatment A and 10 hours treatment B) behaviour was compared from the onset of feeding between the two treatments.

Very low percentages of animals were lying down within the first hour after feeding (figure 3.17). Although there was a significant difference in the increase of lying behaviour between the first and the second hour after feeding. However only a very low percentage of animals lying down was observed in these first two hours. For treatment group B, the increase in percentage of animals lying down increased further in the subsequent hours (see figure 3.11), but these could not be taken consideration for statistical analysis because treatment group A arrived at the destination within the first three hours after feeding.

Concurrently to the increase in percentage of animals lying down, the percentage of animals eating decreased during the first two hours after feeding (figure 3.19). The most interesting finding was however, that the total percentage of animals eating was significantly higher for the treatment group B  than for treatment group A (figure 3.18). This is interesting because heifers in group A had a longer travelling time before feeding and were fed at a 'normal' time (compared with baseline recordings prior to the journey). Hence, the question arises why these animals were less motivated to eat? Several aspects can be considered. It could have been that these animals had received extra food during the daytime hours prior to the journey. Unfortunately, the feeding regime and the amount of food that the heifers received prior to departure were not registered, so this reasoning cannot be verified. Another incentive could have been that the heifers were too exhausted to eat after 14 hours of travelling. Since it was not possible to study behaviour after unloading, it can be hypothesised that 14 hours of travel may have been too demanding. On the other hand, all heifers assessed after unloading were in good condition and not showing signs of extreme exhaustion at a first inspection (see table 3.1). As mentioned earlier, all animals (both treatments) were lying down and ruminating soon after unloading and final clinical assessment (see figure 4.3).

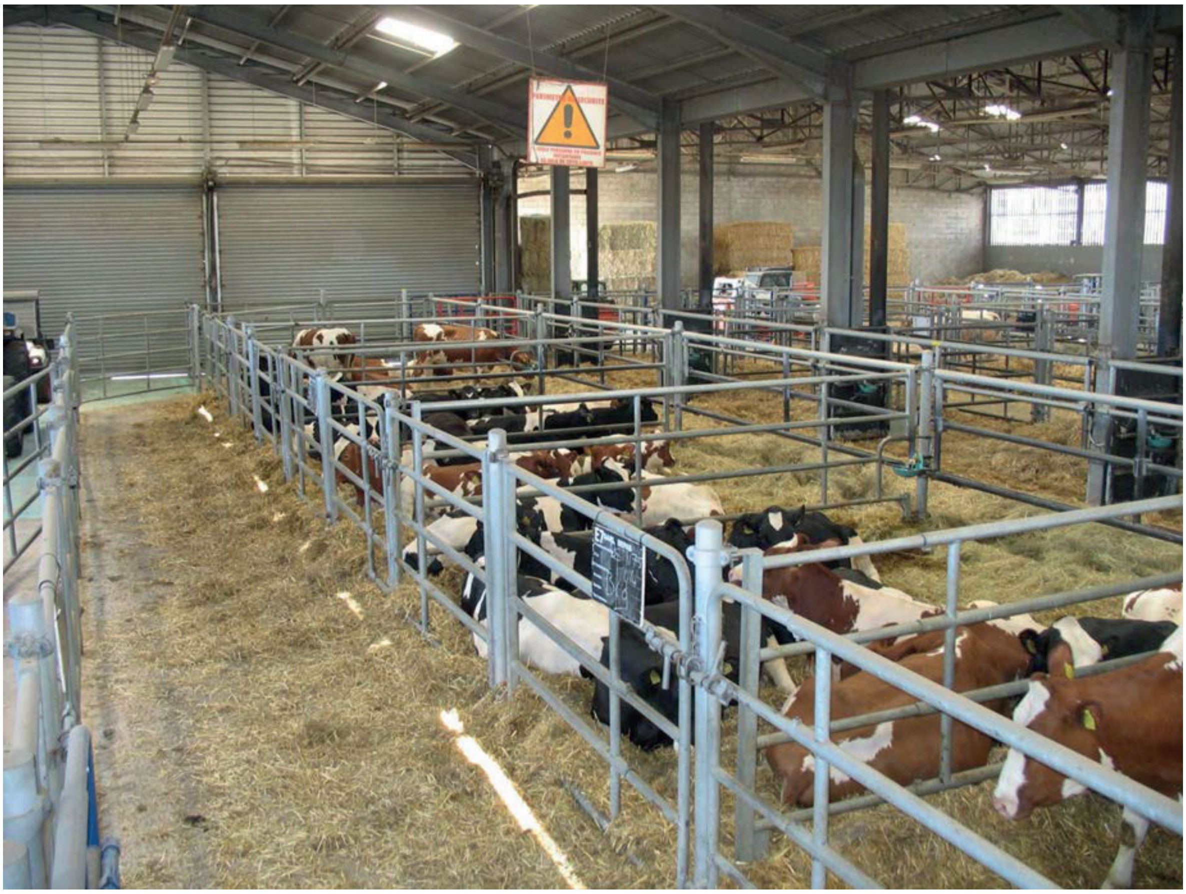

***Figure 4.3*** *An half hour after unloading, heifers from both treatment groups were lying down and ruminating*

The fourth driving block started after feeding for treatment group A. In contrast, heifers in treatment group B, fed in the evening, remained in the vehicle for the 9-hour resting period. Figure 3.20 visualizes this effect for the percentage of animals ruminating, which increased significantly in treatment group B. Therefore, it is hypothesised that provision of a stationary environment immediately after feeding has a positive effect on rumination patterns in heifers.

## 4.3 Conclusions

This type of field studies do inevitably involve constraints such as an inability to standardize external factors  resulting in difficulties in interpretation of the results. Therefore, the results of the study should be interpreted with care and regarded as hypothetical. Accordingly, the conclusions may be laid down as follows:

The results of the study did not show clear evidence that heifers transported (under Cattle Cruiser conditions) with a 9-hour overnight stop had a profound increase in welfare compared to heifers transported with an one-hour stop after 14 hours of transport. However, it was shown that the 9-hour stop overnight had important advantages for animal welfare:

- After feeding, the rumination activity during the 9-hour resting period on the vehicle was similar (duration and peak) to the rumination pattern in the home environment prior to the journey.

- Providing a stationary environment during the night and during several hours after feeding has a positive effect on both eating duration and rumination pattern. Attention must be paid to provide sufficient space per animal to be able to lie down during the night.

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# Appendix 1 Journey information

**Table Appendix 1** Information of the six journeys (departure date, total distance covered, time of departure and arrival, water consumption and mean compartment temperature)

| | Departure Date | Treatment | Total distance (in km) (official journey log) | Time of departure (logbook drivers) | Time of arrival (logbook drivers) | Water consumption in total liters | Mean temperature in the vehicle (measured by Kleventa, in °C) |
|---|---|---|---|---|---|---|---|
| **1** | June 6th 2013 | A | 1136 | 17:05 | 10:15 | 387 | 17.2 ± 2.4 |
| | | B | 1184 | 08:00 | 12:45 | | 21.1 ± 2.5 |
| **2** | September 19th 2013 | A | 1162 | 18:05 | 11:45 | 180 | 18.2 ± 3.2 |
| | | B | 1204 | 09:00 | 12:15 | 414 | 20.8 ± 2.9 |
| **3** | January 30th 2014 | A | 1156 | 19:05 | 12:24 | 315 | 09.8 ± 2.7 |
| | | B | 1196 | 09:01 | 13:05 | | 14.1 ± 2.8 |
| **4** | February 13th 2014 | A | 1156 | 19:05 | 11:40 | | 10.2 ± 2.9 |
| | | B | 1174 | 09:16 | 13:00 | 720 | 13.3 ± 2.3 |
| **5** | May 16th 2014 | A | 1163 | 19:05 | 11:30 | | 17.2 ± 3.3 |
| | | B | 1195 | 09:02 | 11:50 | | 19.6 ± 2.2 |
| **6** | May 30th 2014 | A | 1152 | 19:05 | 11:30 | 135 | 17.7 ± 3.3 |
| | | B | 1171 | 09:05 | 11:30 | | 21.3 ± 2.3 |

# Appendix 2 Specification of the Cattle Cruiser

## DEFINITIE CATTLE CRUISER

*Bedacht en in praktijk gebracht door H van Dommelen, Rietveld 47, 3443 XB Woerden, NL*

| | |
|---|---|
| *Afmetingen:* | *Inwendige lengte op zwanenhals, 4,00 m, achter 9,70 m, boven verdeeld in 3, onder in 2 vakken. Inwendige breedte 2,47 m. Inwendige hoogte bovenin 180 cm, onder ook, behalve voorin, daar 90 cm voor kistruimte.* |
| *Wanden:* | *Rondom geïsoleerde wanden* |
| *Ventilatie:* | *Aan linkerzijde, per laag hoog ca. 40 cm, aan binnenzijde voorzien van geperforeerde plaat. Ventilatie startend ca 130 cm boven vloer, afte dichten d.m.v. brede, elektrisch bediende, alucobond schuiven. Aan rechterzijde geen ventilatie, doch alleen ventilatoren.* |
| *Ruiven:* | *Aan rechterzijde tussen de ventilatoren openklappende ruiven van 70 cm hoogte. Kleppen blijven op 45° open staan, kunnen echter ook 180° openklappen. Onderkant ruiven op ca 100 cm. Achter de ruiven verticale spijlen. Een deel van deze spijlen vormt een deurtje voor ruimte toegang tot elk vak, ter plaatse van de drinkbakken.* |
| *Vloer:* | *Bestaande uit 40 mm aluminium plankprofielen met uitgefreesd ribbenprofiel, in lengte gelegd. Achter een 5 cm hoge drempel. Afwatergaten links en rechts achter, afte sluiten met stop.* |
| Afloop: | Achterste 4,6 m van bovenlading een afloop, met aan het eind een schamierend aangebrachte rechtop staande brug van 160 cm. |
| Klimrek: | Afloop voorzien van opklapbaar klimrek |
| Kap: | 40 mm geïsoleerde dakplaat |
| Achterzijde: | Onderste 80 cm een omlaag schamierende klep. Daarboven twee geïsoleerde mega concept achterdeuren. |
| Tussenhekken: | Bovenin 2 stuks, onderin 1 stuks tussenhekken uit dubbelwandig aluminium. Hekken zijn 130 cm hoog en blijven 30 cm van de grond vrij i.v.m. dikke laag stro. |
| Eindhekken: | Onder een 2-delig, en bovenin een 1-delig aluminium eindhek uit dubbelwandig aluminium |

# DEFINITIE CATTLE CRUISER

*Bedacht en in praktijk gebracht door H van Dommelen, Rietveld 47, 3443 XB Woerden, NL*

Drinkinstallatie: T.p.v. zwanenhals en in rechter achterhoek wordt een drinkwaterzuil ingebouwd, bestaande uit tanks met een inhoud van ca 600 resp. 300 liter, met daaronder een elektrische pomp met waterfilter en ingebouwde drinkbakken op 80 cm hoogte met suevia.nl staafventielen type 19R voor eenvoudig schoonmaken. De bakken zijn halve maanvormig, zodat 2 koeien tegelijk kunnen drinken en zo geplaatst dat elk vak de beschikking heeft over 1 drinkbak. Diepte drinkbakken ca 10 cm, vrije hoogte boven drinkbakken ca 40 cm. Ontluchting van tanks naar buitenzijde oplegger zodat van buiten zichtbaar is wanneer de tanks vol zijn, door overstromen ontluchting.

Zoutblokken: Bovenin mineralenblokken geplaatst tussen 1e en 2e vak, zo hoog mogelijk. Onderin blokken op wielbakken rechts.

Ventilatoren: In de rechter zijwand, in totaal 10 stuks ventilatoren. Luchtopbrengst 1200 $m^3$ lucht per uur. Ventilatoren zijn aangesloten op KVM registratie unit voor automatische aansturing waarbij elk vak apart wordt aangestuurd door zijn eigen sensor. Ventilatoren blazen standaard naar buiten, zijn echter om te schakelen. Wanneer de temperatuur boven een ingestelde waarde komt, worden de ventilatoren traploos bijgeschakeld.

Stroomvoorziening: Accu's welke geladen worden door de trekker. Tevens een acculader, waardoor de accu's met externe voeding (op de boot) opgeladen kunnen worden.

Verlichting: In plafond ingebouwde LED binnenverlichtingslampen, twee per vak.

Kistruimte: Onderste gedeelte op zwanenhals ingericht als kistruimte voor opbergen hooi.

Ladder: Een ladder met bordes.

Reinigen: Een op eigen kracht werkende hogedrukspuit

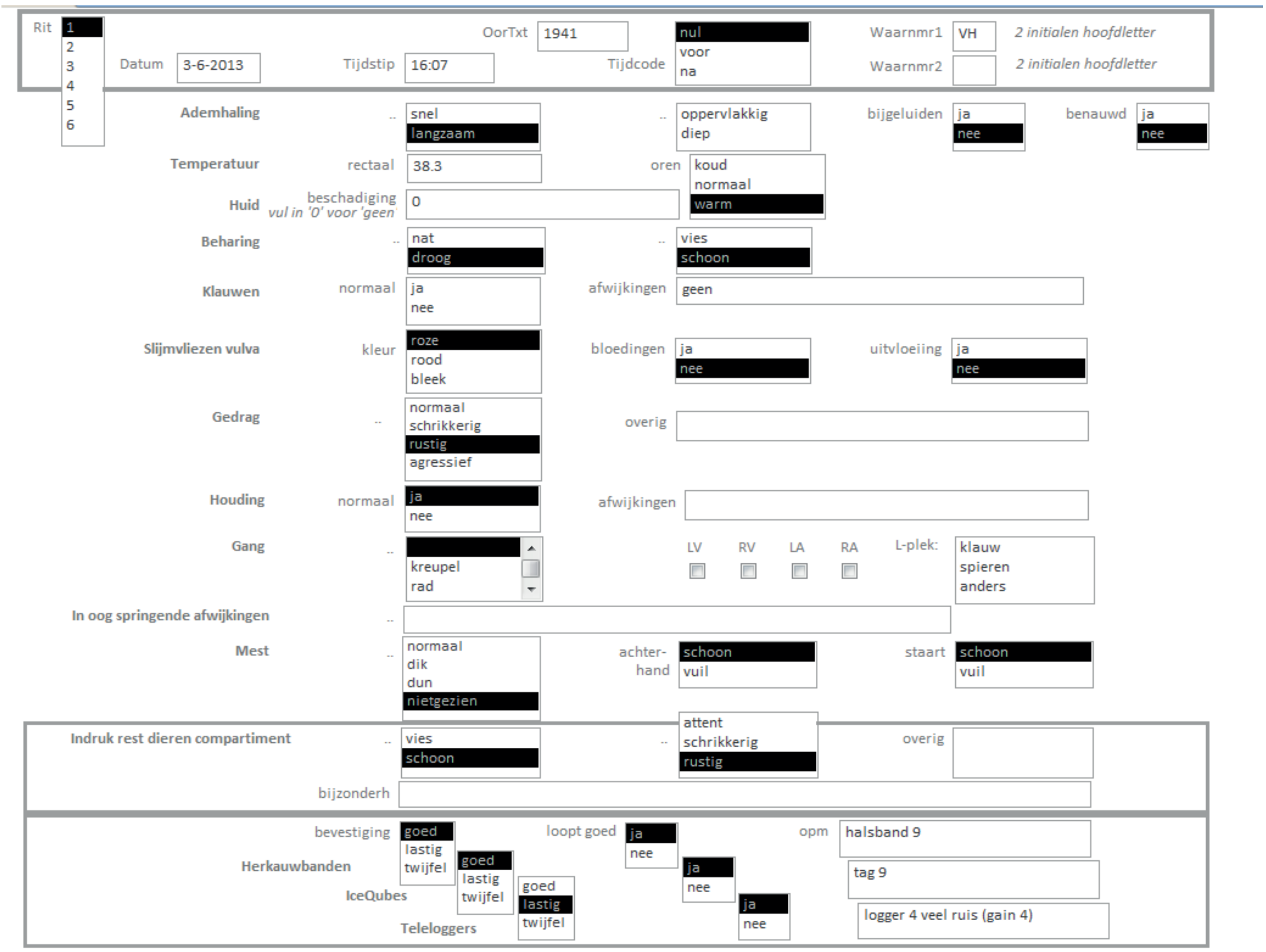
# DEFINITIE CATTLE CRUISER

*Bedacht en in praktijk gebracht door H van Dommelen, Rietveld 47, 3443 XB Woerden, NL*

Temperatuurregistratie systeem type KVM volgens EG richtlijn 112005, transport langer dan 8 uur

Uitvoering: Het KVM systeem bestaat uit een MU (Main Unit), waarop 5 sensoren worden aangesloten. Sensoren in het midden van elk vak. De sensoren geïsoleerd van de vloer, zodat ze de temperatuur van de ruimte meten en niet de temperatuur van de vloer waar ze in gemonteerd zijn. De meetwaarden alsmede de gevolgde route en de toestand van de laadklep (open-dicht) met datum en tijdsaanduiding worden automatisch opgeslagen op een multimedia kaart. Dataoverdracht via het GSM telefoonnetwerk en internet, waardoor de oplegger real-time is te volgen. In de cabine wordt een RHI display unit geplaatst, waarop de temperaturen afgelezen kunnen worden en de ingestelde waarden gewijzigd. Alarmen worden per SMS bericht aan gewenste mobiele telefoon(s) doorgegeven. Tevens gaat de contourverlichting knipperen en een sirene af, als er alarm is, zodat omstanders worden gealarmeerd.Example of clinical scoring form

# Appendix 3 Baseline recordings lying down

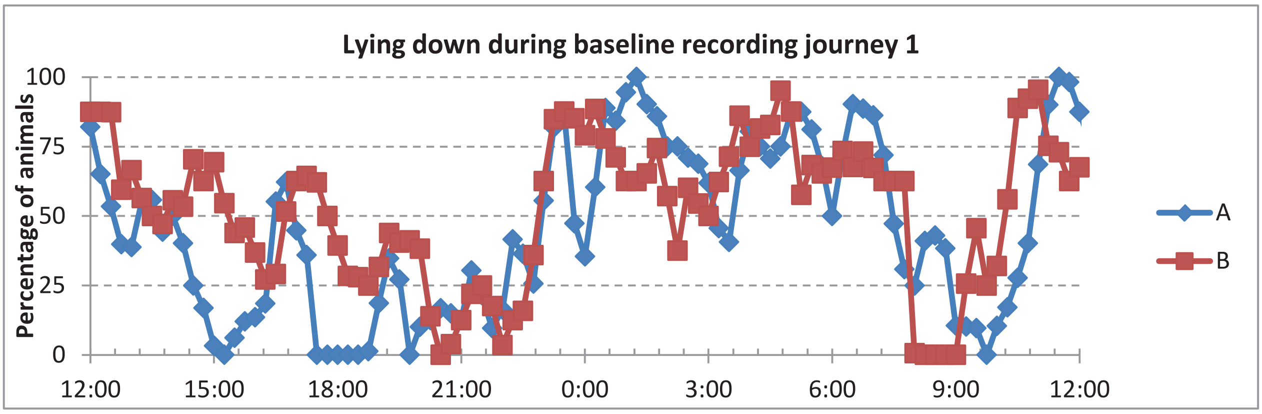


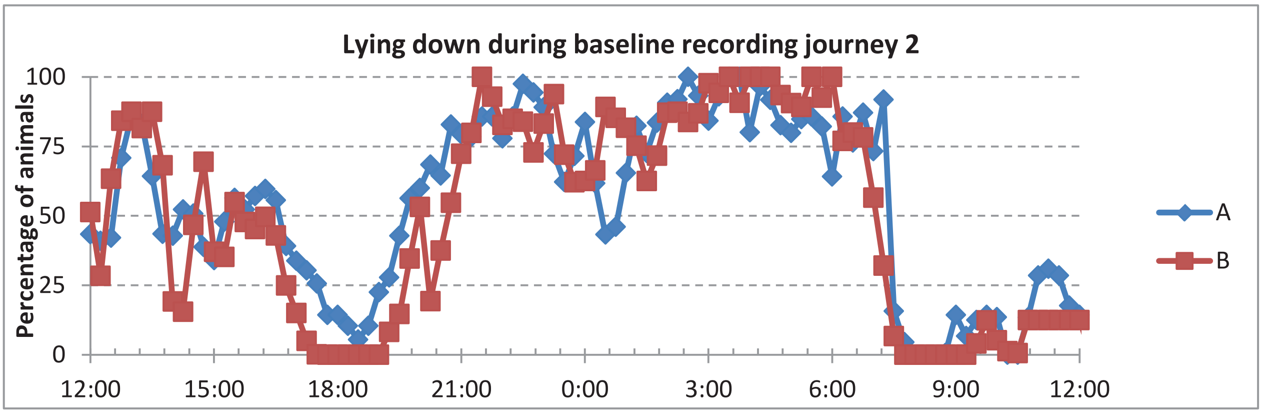
Rit: 1 / 2 / 3 / 4 / 5 / 6
Datum: 3-6-2013
OorTxt: 1941
Tijdstip: 16:07
Tijdcode: nul / voor / na
Waarnmr1: VH — *2 initialen hoofdletter*
Waarnmr2: — *2 initialen hoofdletter*

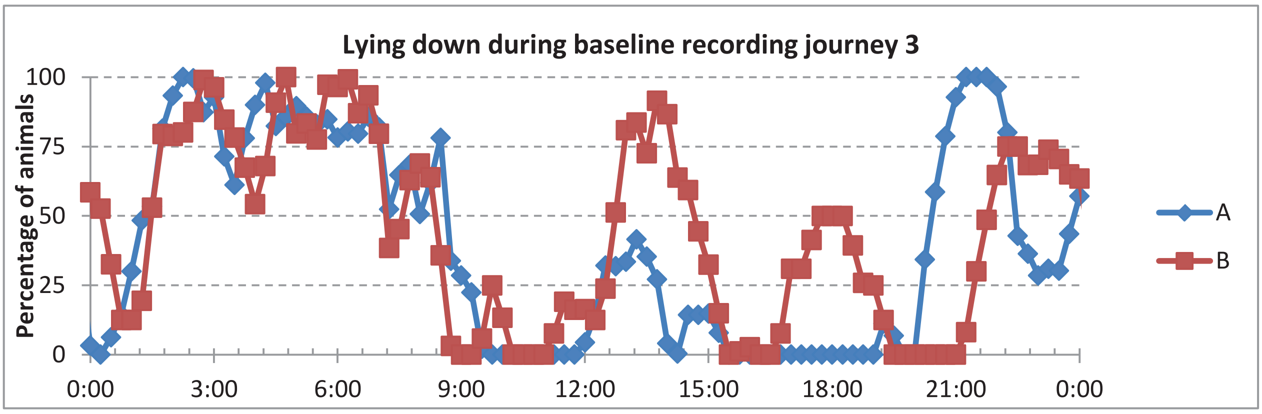
**Ademhaling** .. snel / langzaam; .. oppervlakkig / diep; bijgeluiden: ja / nee; benauwd: ja / nee
**Temperatuur** rectaal: 38.3; oren: koud / normaal / warm
**Huid** beschadiging *vul in '0' voor 'geen'*: 0
**Beharing** .. nat / droog; .. vies / schoon
**Klauwen** normaal: ja / nee; afwijkingen: geen
**Slijmvliezen vulva** kleur: roze / rood / bleek; bloedingen: ja / nee; uitvloeiing: ja / nee
**Gedrag** .. normaal / schrikkerig / rustig / agressief; overig:
**Houding** normaal: ja / nee; afwijkingen:
**Gang** .. kreupel / rad; LV, RV, LA, RA; L-plek: klauw / spieren / anders
**In oog springende afwijkingen** ..
**Mest** .. normaal / dik / dun / nietgezien; achter-hand: schoon / vuil; staart: schoon / vuil
**Indruk rest dieren compartiment** .. vies / schoon; .. attent / schrikkerig / rustig; overig:
bijzonderh:
bevestiging: goed / lastig / twijfel; loopt goed: ja / nee; opm: halsband 9
**Herkauwbanden**: goed / lastig / twijfel; ja / nee; tag 9
**IceQubes**: goed / lastig / twijfel; ja / nee; logger 4 veel ruis (gain 4)
**Teleloggers**

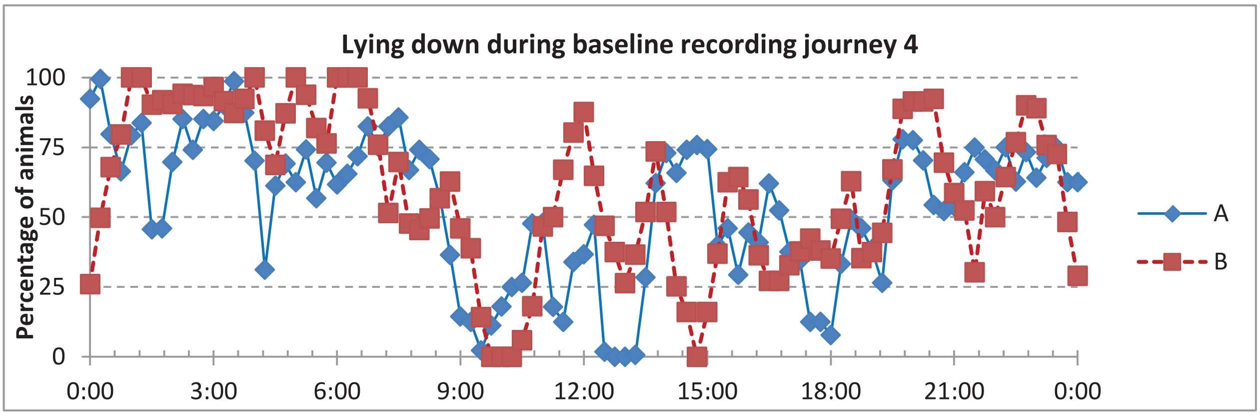
# Appendix 4 Baseline recordings lying down

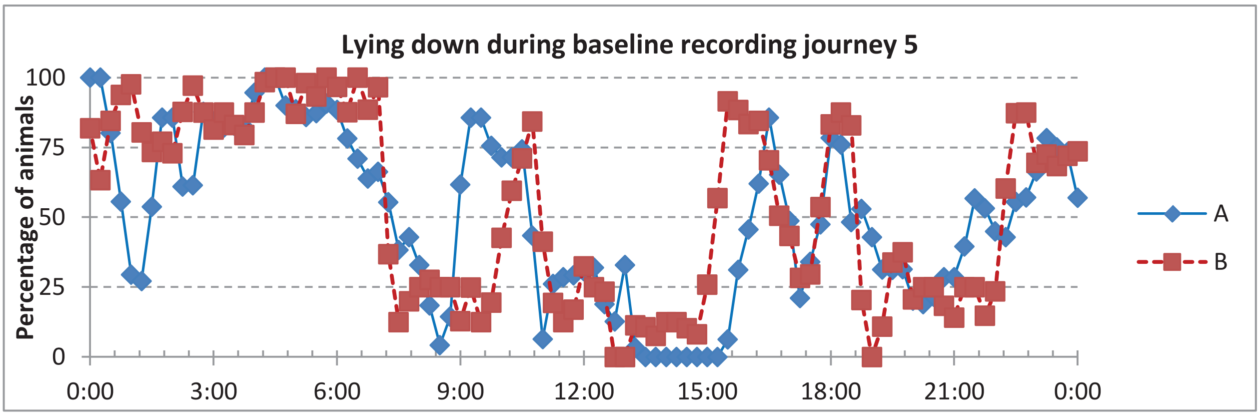
Figure Appendix 4. Percentage of animals per treatment A (n=8) and B (n=8) that is lying down during a 24 hour baseline recording within 48 hours preceding the day of departure. 24 hour baseline recordings of journey 3-6 were started later (0:00 midnight instead of 12:00 noon) because of interference with activities in the stable that could affect baseline behaviour.

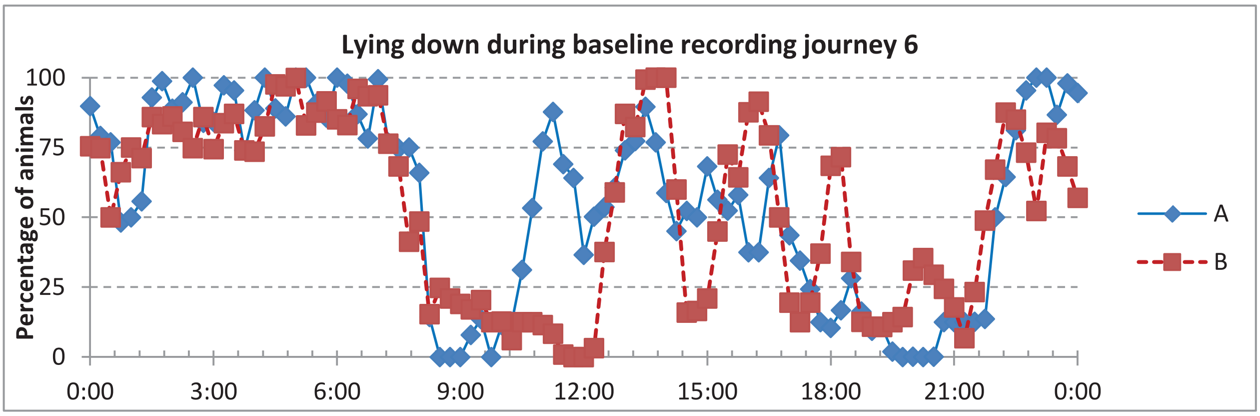


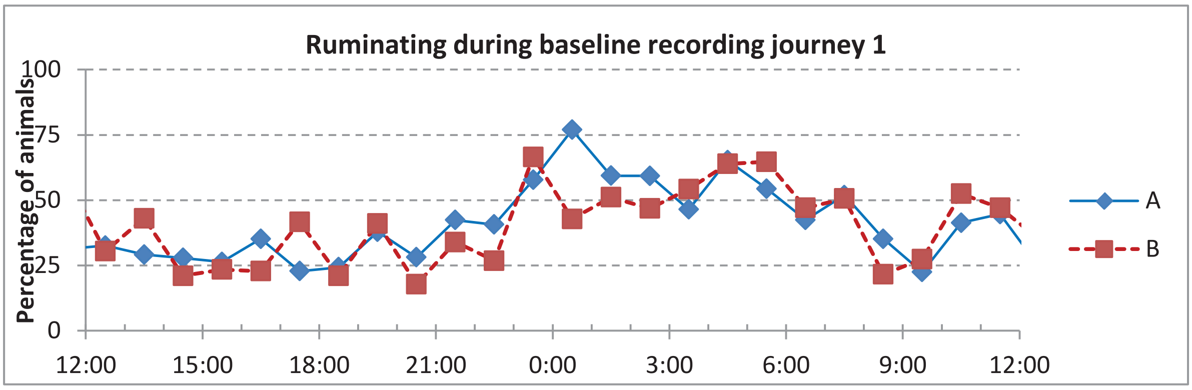

Lying down during baseline recording journey 4
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

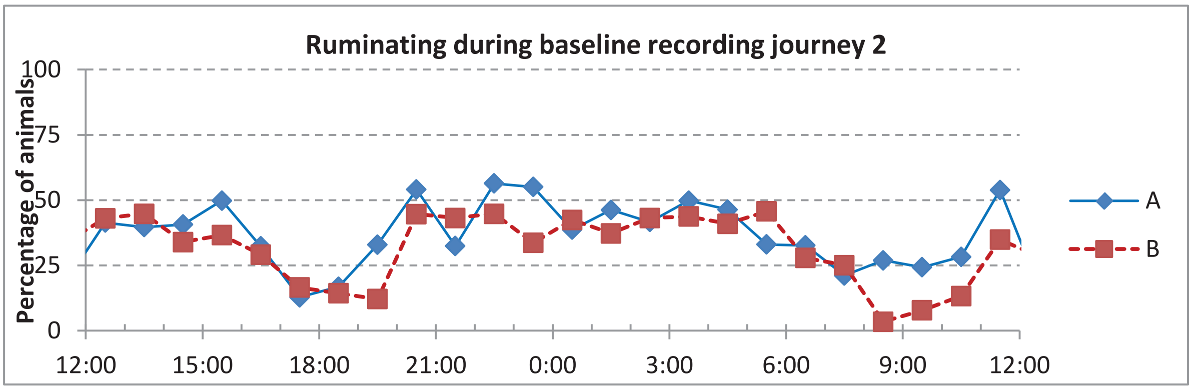

Lying down during baseline recording journey 5
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

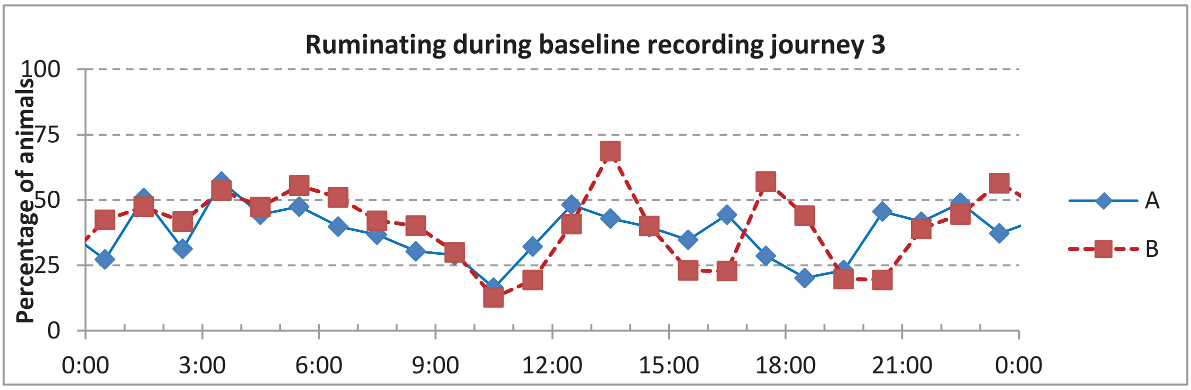

Lying down during baseline recording journey 6
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

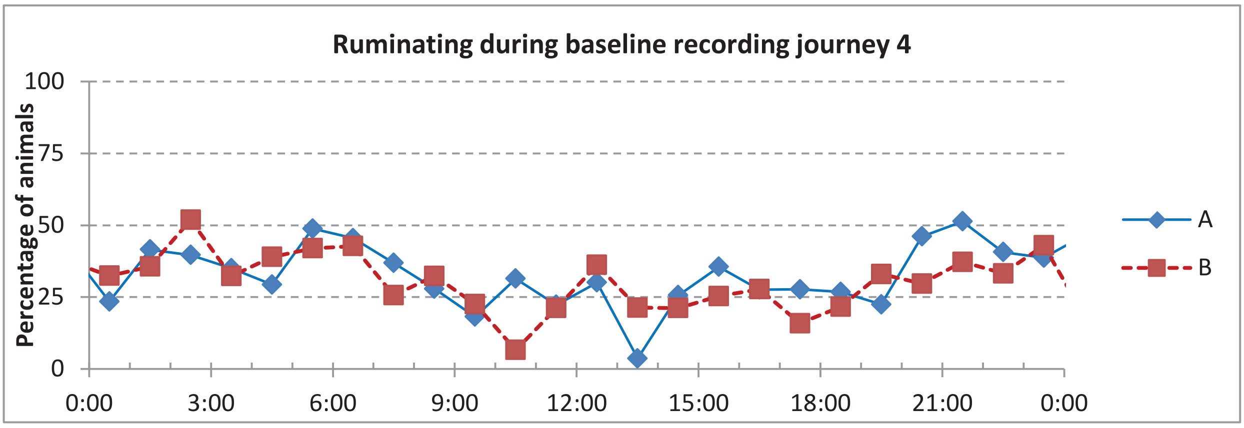
# Appendix 5 Baseline recordings ruminating

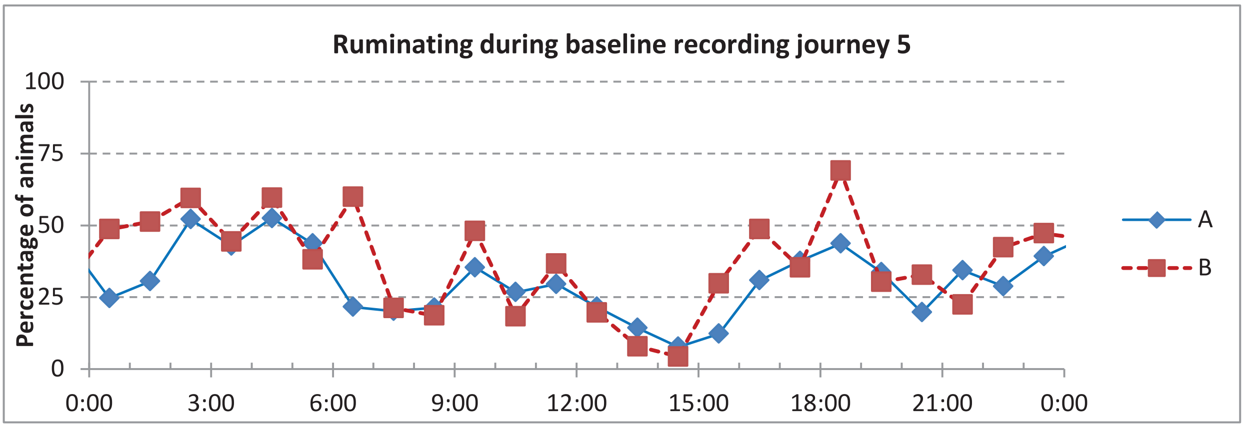
Figure Appendix 5 Percentage of animals per treatment A (n=8) and B (n=8) that is ruminating during a 24 hour baseline recording within 48 hours preceding the day of departure. 24 hour baseline recordings of journey 3-6 were started later (0:00 midnight instead of 12:00 noon) because of interference with activities in the stable that could affect baseline behaviour.

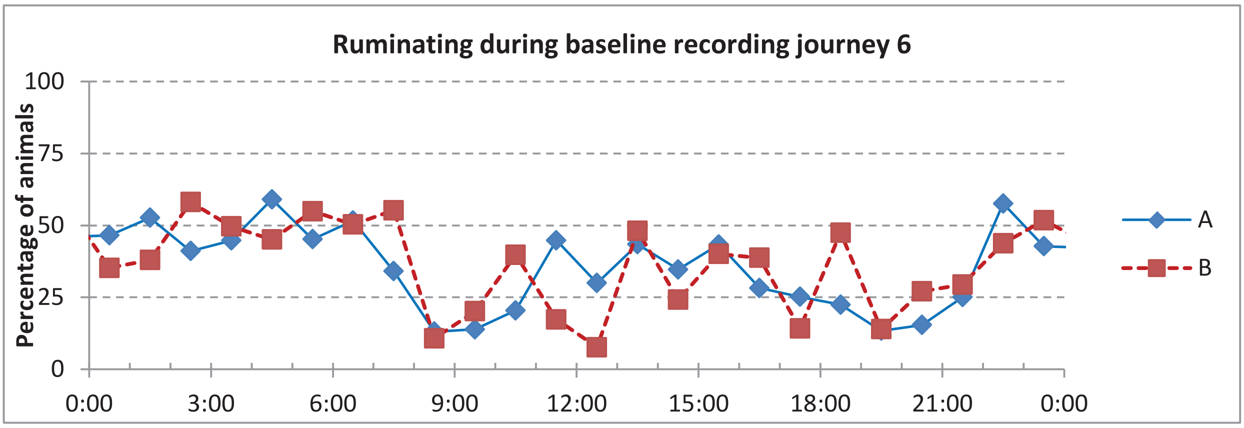


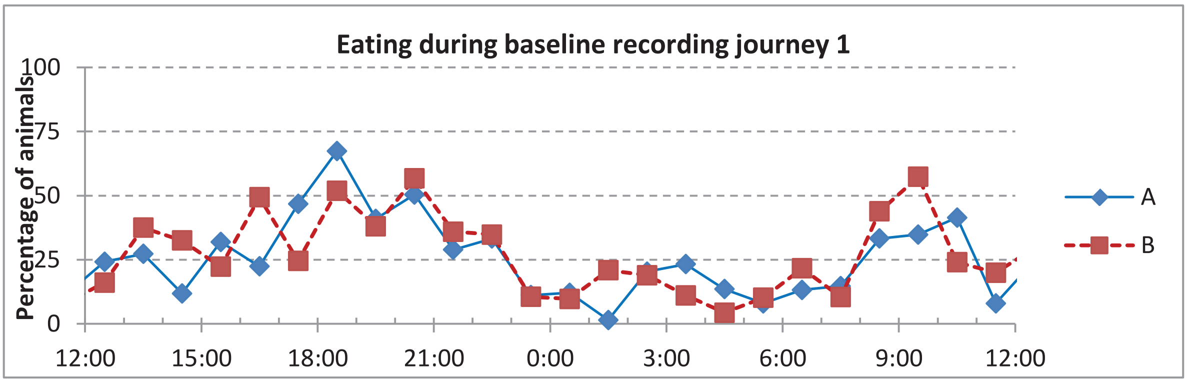

Ruminating during baseline recording journey 4
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

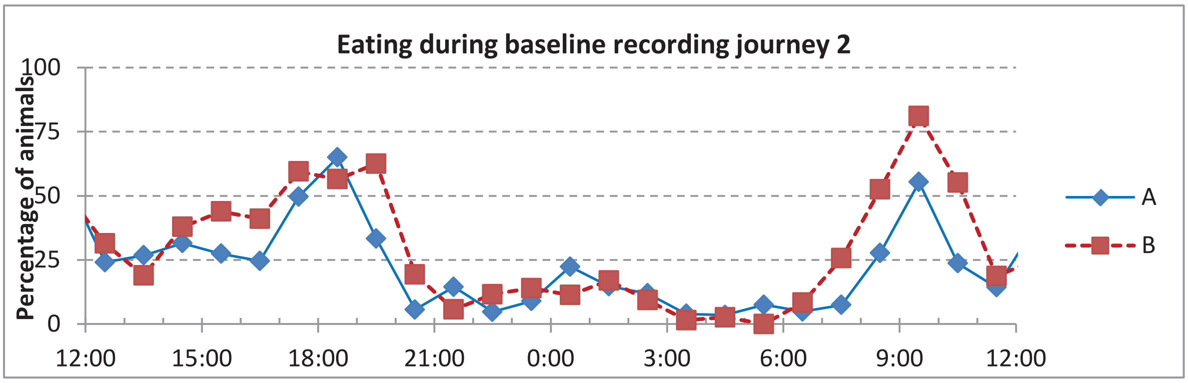

Ruminating during baseline recording journey 5
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

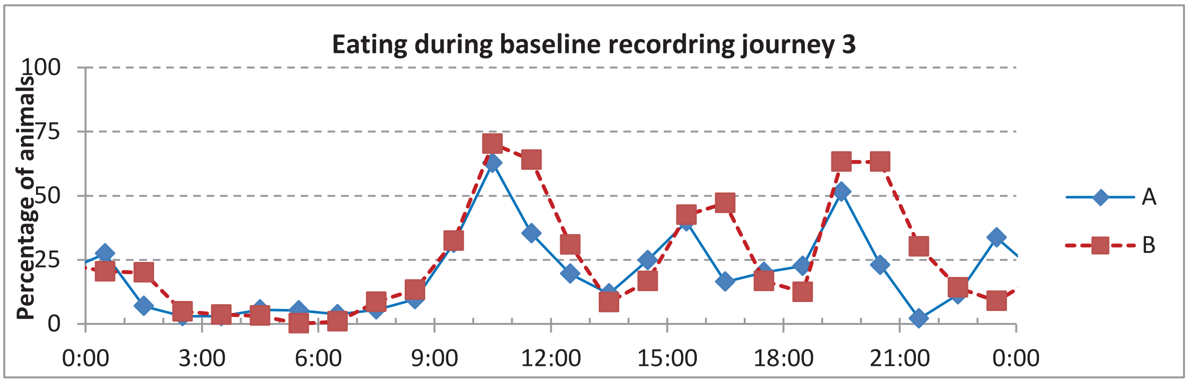

Ruminating during baseline recording journey 6
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

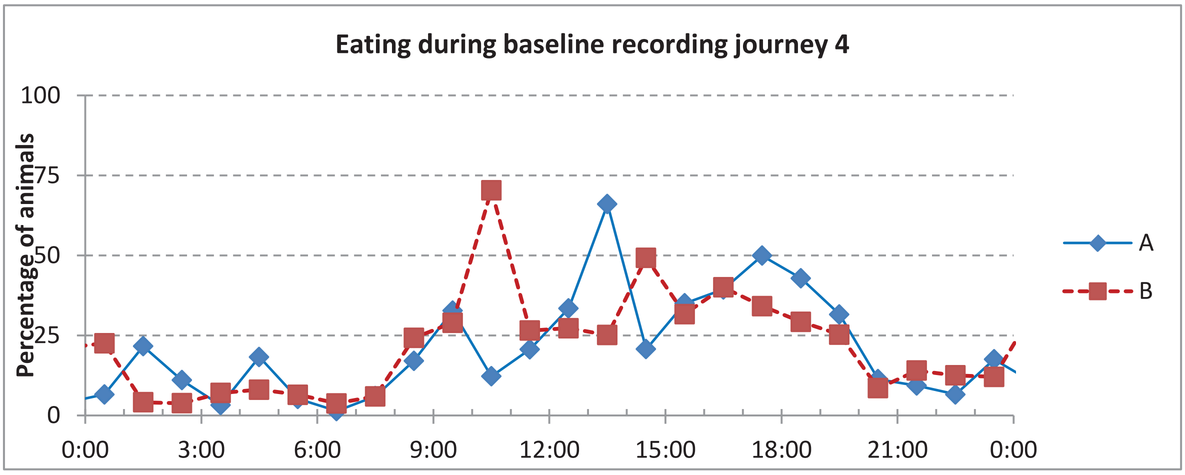
# Appendix 6 Baseline recordings eating

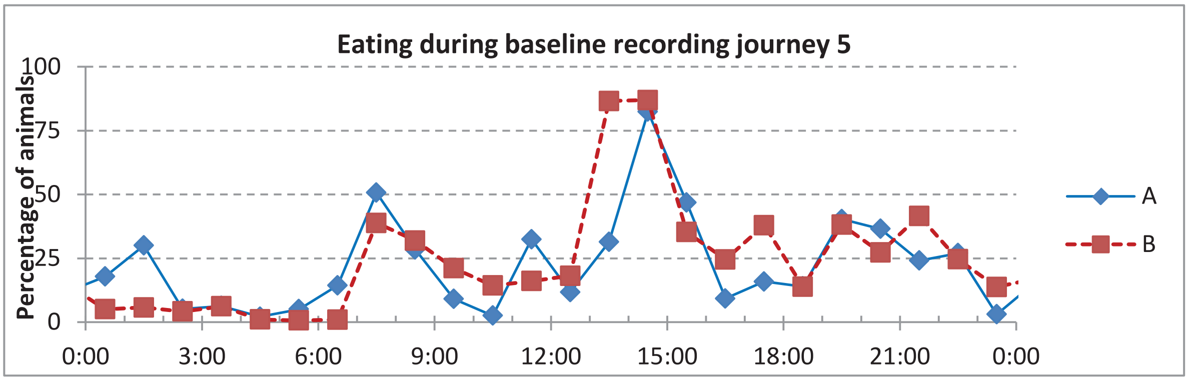
Figure Appendix 6 Percentage of animals per treatment A (n=8) and B (n=8) that is eating during a 24 hour baseline recording within 48 hours preceding the day of departure. 24 hour baseline recordings of journey 3-6 were started later (0:00 midnight instead of 12:00 noon) because of interference with activities in the stable that could affect baseline behaviour.

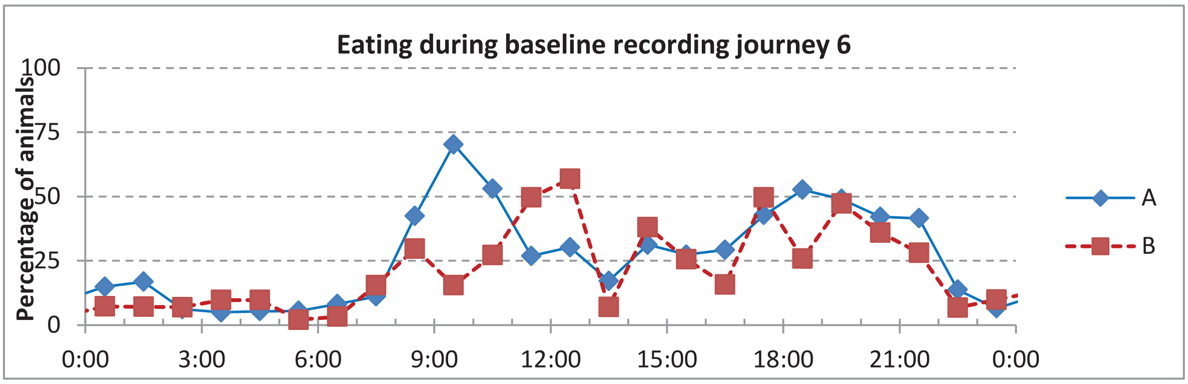



Eating during baseline recording journey 4
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B


Eating during baseline recording journey 5
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B


Eating during baseline recording journey 6
Percentage of animals
100
75
50
25
0
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
0:00
A
B

# Appendix 7 Examples of lying down patterns individual animals

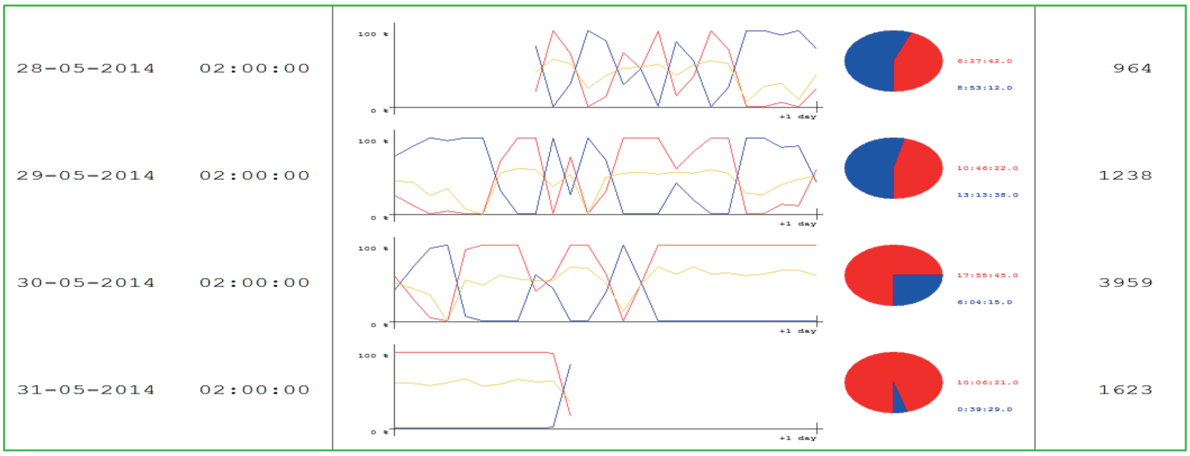



***Figure Appendix 7.1*** *Lying down and standing of one heifer in treatment group A that did not lie down during the whole journey. Red line is standing, blue is lying down. Journey starts on the third block (on May 30th 2014)*

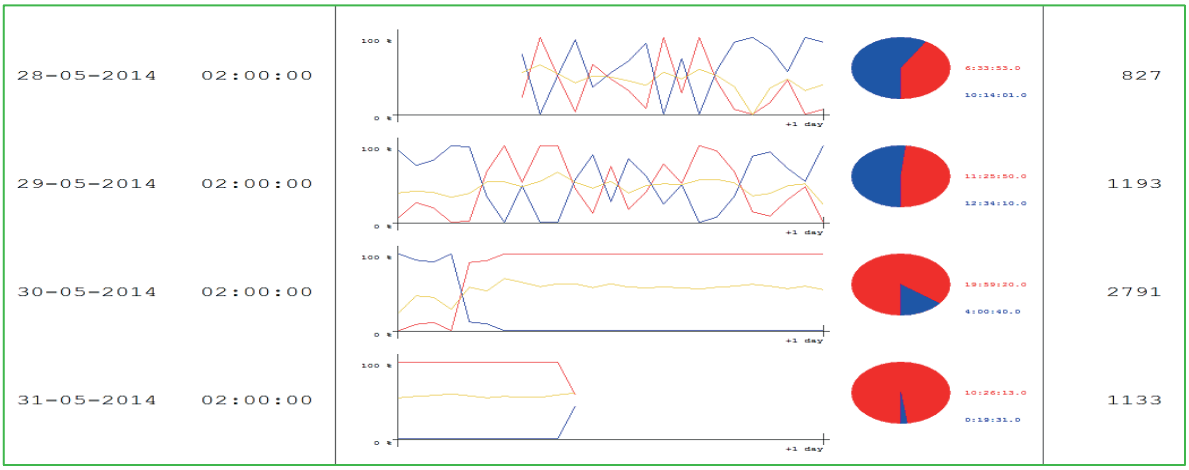



***Figure Appendix 7.2*** *Lying down and standing of one heifer in treatment group B that did not lie down during the whole journey, including the 9 hour overnight stop. Red line is standing, blue is lying down. Journey starts on the third block( on May 30$^{th}$ 2014)*

# Appendix 8 Examples of eating and ruminating patterns individual animals

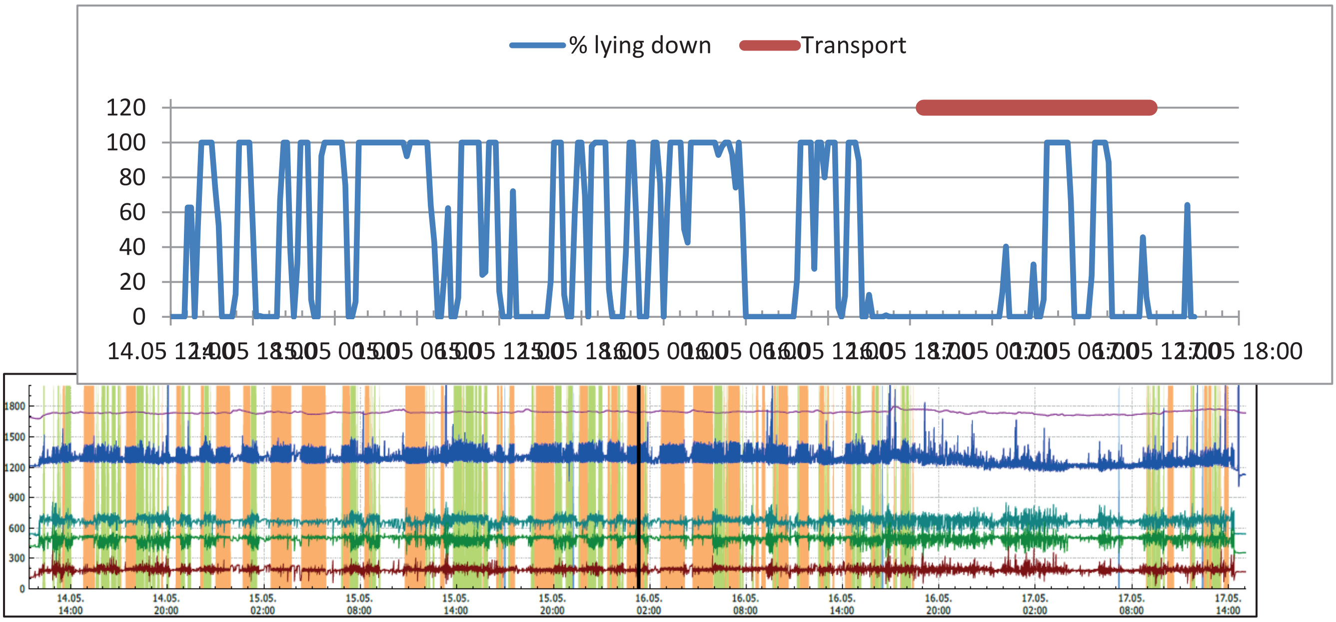



***Figure Appendix 8.1*** *Ruminating and eating of one heifer in treatment group A with hardly eating and ruminating during the journey*

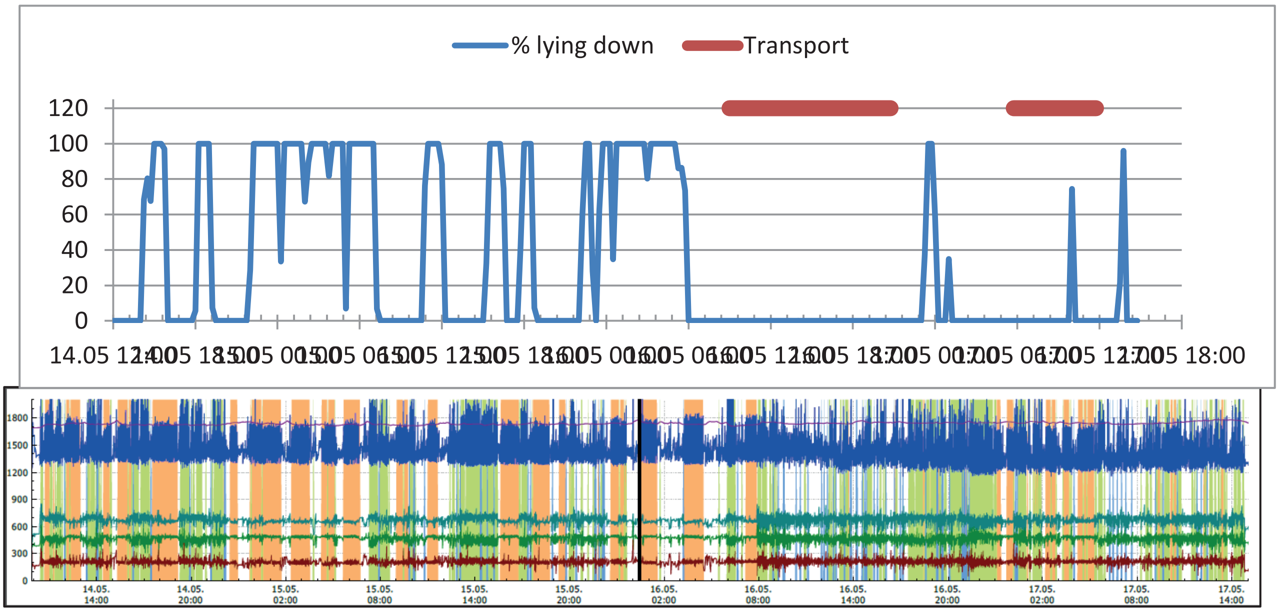



***Figure Appendix 8.2*** *Ruminating and eating of one heifer in treatment group B with profound activities of eating and ruminating during the journey and during the 9-hour overnight stop*

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