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Transport Conditions of Fattening Pigs from Farm to Slaughterhouse

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Abstract
An investigation of travelling conditions of slaughter pigs during 8 long (>8h) journeys across Germany. Animals were transported at two loading densities. Observations of physiological (heart activity , blood parameters, body temperature) and behavioural responses (posture, fighting) were made together with registration of environmental aspects (including indoor and outdoor temperature, wind speed, humidity, weather conditions). Driving conditions were also registered.

Keywords
Long transport, slaughter pigs, loading density, physiological and behavioural responses, conditions of transport.

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Transport Conditions of Fattening Pigs from Farm to Slaughterhouse

Transport of pigs for more than 8 hours at two space allowances

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Preface

A recent workshop (6-7 April 2008; Schiphol NL) organized by the Dutch ministry for the Economy, Agriculture and Innovation (EL&I) in co-operation with Animal Sciences Group (Wageningen UR) identified the critical risk factors during transportation of livestock. One of the most important risk factors is the amount of living space (loading density) available during transit. It was established that there is insufficient information available concerning transport conditions for pigs.
Therefore a policy support project was initiated by EL&I under BO-07-011-055 “Verbetering transport condities” to investigate and advise on (long) transport conditions of pigs.

Samenvatting

Hoe dieren om kunnen gaan met transport omstandigheden hangt af van deze transport omstandigheden, van de leeftijd en fitheid van dieren en van hun mogelijkheden om te reageren op deze omstandigheden. De omstandigheden waaraan dieren tijdens transport worden blootgesteld hangt sterk af van de transportwagen, rijgedrag, ventilatie en van de weersomstandigheden. Het effect van deze wisselende omstandigheden op het welzijn van dieren is niet voor alle dieren duidelijk. Algemeen wordt gesteld dat alle dieren tijdens transport moeten kunnen staan en liggen wanneer ze daar behoefte aan hebben. Dieren moeten daarom voldoende stahoogte tot hun beschikking hebben om te kunnen staan in een natuurlijke houding en voldoende bewegingsvrijheid hebben om te kunnen drinken. Daarnaast moet er voldoende ruimte in de transportwagen zijn om goede ventilatie te garanderen. Een van de belangrijkste risico factoren voor dierenwelzijn tijdens transport is de beladingsgraad (van Reenen et al., 2008) en er is vastgesteld dat er niet voldoende informatie is hoe varkens om kunnen gaan met verschillende transportcondities vooral gedurende middellange transporten.

Op verzoek van het ministerie van Economische Zaken, Landbouw en Innovatie is onderzoek gedaan tijdens het transport van varkens gedurende langere afstanden (langer dan acht uur). Dit onderzoek omvat acht transporten over de weg met vleesvarkens die bij twee bezettingsgraden vervoerd werden. De varkens werden in de periode juni tot oktober 2010 van een boerderij in Sleeswijk-Holstein getransporteerd door Duitsland naar een slachterij in het district Dahme-Spreewald in de deelstaat Brandburg in het oosten van Duitsland. Tijdens het vervoer werd vooral gekeken naar dierenwelzijn. Verschillende factoren die het welzijn van de varkens beïnvloeden werden geregistreerd, zoals klimaat (temperatuur, windsnelheid, luchtvochtigheid) en factoren die met het transportmiddel te maken hebben (trillingen en rijstijl). Ventilatie, leef- en sta-ruimte, of vrije bewegingsruimte rondom het dier en erboven, zijn belangrijke factoren die de leefomgeving gedurende het transport beïnvloeden. Varkens zijn erg gevoelig voor transportomstandigheden, maar kunnen zich aanpassen aan nieuwe situaties. Fysiologische indicatoren, zoals cortisol in het bloed, proteïne, albumine, creatine kinase (CK), witte bloedlichaampjes, Hematocriet (Ht) en Hemoglobine (Hb) zijn voor vertrek van het bedrijf en na aankomst bij het slachthuis gemeten. Andere indicatoren zoals lichaamstemperatuur (ibutton thermologger) en hartslag (telemetrisch logger) werden gedurende elk transport bij acht proefdieren gemeten. Deze dieren bevonden zich in vier compartimenten aan de voorkant op het onderste en bovenste dek en in de achterste compartimenten van de vrachtwagen. Het gedrag van de varkens werd gemonitord door gebruik van camera's in het voorste en achterste deel van zowel de boven- als onderste compartiment. Het is bekend dat de duur van het transport en de plaats in het transportmiddel het gedrag van dieren beïnvloeden (Averios et al 2007, Warriss 1998). Plaatsing in de wagen draagt bij aan stress en sterfte vaak in relatie met een hoge bezettingsgraad. Het doel van het onderzoek was gericht op het opstellen van algemene voorwaarden voor transport van varkens en of de norm van 235 kg/m^2 aangepast moet worden. De beoordeling van het aanpassingsvermogen van de varkens is gebaseerd op de eerder genoemde parameters gemeten tijdens de acht transporten onder commerciële transport omstandigheden.

De resultaten laten zien dat tijdens het inladen de lichaamstemperatuur meer steeg in de groepen met een normale bezetting dan in de groepen met een lage bezetting. Omdat alle groepen dieren op dezelfde manier werden behandeld, lijkt de stijging veroorzaakt te worden door een verhoging in activiteit of stress in combinatie met minder ruimte in de wagen. Tijdens het transport daalde de lichaamstemperatuur binnen één uur af naar normale waarden, maar bij de hogere bezettingsgraad bleef de lichaamstemperatuur van de dieren iets hoger dan bij een lagere bezettingsgraad. Dit werd ook waargenomen wanneer gekeken werd naar de hartslag. De hartslag nam tijdens het laden aanzienlijk toe. Zodra alle dieren ingeladen waren en het voertuig gesloten was daalde de hartslag naar normale waarden. Hoewel niet significant, bleek de hartslag hoger te zijn in de groepen met een normale bezetting vergeleken met de lage bezetting. Opmerkelijk was de stijging van de hartslag tijdens de lunchpauze van de chauffeur. Kort nadat de vrachtwagen was gestopt, steeg de lichaamstemperatuur in de groep met een normale bezettingsgraad meer dan in de groep met een lage bezettingsgraad. Kort nadat de lichaamstemperatuur begon te stijgen, steeg ook de hartslag. Meer dan 80% van de dieren lag tijdens de pauze en de verwachting was dat de lichaamstemperatuur en hartslag stabiel bleven. Opvallend is dat er tijdens de pauze geen (mechanische) ventilatie was, waardoor de temperatuur in de wagen steeg.

Er wordt aangenomen dat als gevolg van de oplopende omgevingstemperatuur, er behoefte is aan meer ventilatie om de lichaamstemperatuur constant te houden.
Er wordt aangenomen dat vermoeidheid kan worden waargenomen door een verandering van spierenzymen als creatine kinase (CK) en lactaat dehydrogenase (LDH). Veranderingen in aspartaat amino transferase (AST) tijdens het transport worden gezien als indicator voor afbraak van spierweefsel. Een lage bezettingsgraad resulteerde in een lagere CK en AST niveau (trend) in het bloed in vergelijking met een normale bezettingsgraad. Rekening houdend met de langere gevechtsduur en kortere rustperiode bij een normale bezettingsgraad werd ook verwacht dat er bij een dichtere bezettingsgraad meer vermoeide varkens waren dan bij een lagere bezettingsgraad. Kijkend naar het hogere cortisol niveau bij een normale bezettingsgraad en vergeleken met het CK en AST niveau en de kortere rusttijden, is het aannemelijk dat dieren bij een normale bezettingsgraad meer stress ervaren dan bij een lage bezettingsgraad.

Concluderend kunnen we spreken van verschillen tussen de transporten en tussen de bezettingsdichtheid, maar gezien de grote verschillen tussen individuele transporten is het niet mogelijk een conclusie te trekken voor dierenwelzijn ten gunste van de bezettingsgraad. Echter, het verschil in vecht- en rustgedrag, hartslag en bloedwaarden lijkt aan te geven dat varkens met voldoende leefruimte beter in staat zijn te reageren op verschillende transportcondities.

Deze conclusies zijn gebaseerd op:

- Vecht gedrag wordt beïnvloed door bezettingsgraad, fase van het transport en groepssamenstelling (onbekende reisgenoten).
- De hartslag en lichaamstemperatuur namen toe tijdens het laden. Dit duidt erop dat dit de meest stressvolle periode tijdens het transport is voor de dieren.
- De verschillen in niveaus van cortisol, CK en AST zijn niet significant verschillend tussen de bezettingsgraden. Er zijn dus geen duidelijke indicaties van verschillen in vermoeidheid door meer activiteit of minder rust en meer vechten gezien bij een hogere bezettingsgraad.
- Bij de lagere bezettingsgraad lag een groot aantal varkens tijdens het transport, terwijl bij een normale bezettingsgraad bijna geen enkel dier lag. Hieruit wordt geconcludeerd dat varkens binnen drie tot vier uur na vertrek gaan liggen en rusten wanneer er voldoende leefruimte wordt aangeboden.
- Waterverbruik per dier verschilde nauwelijks tussen de bezettingsgraden. Tijdens het vervoer maakten de dieren gebruik van de drinkwaterfaciliteiten vanaf de aanvang van een rit.

Langere transporttijden (e.g. > acht uur) vormen een risico waarbij varkens hun fysiologische grens overschrijden en hun gedrag (negatief?) aanpassen als gevolg van stress. Hiervoor zijn slechts beperkte management maatregelen nodig zoals het gebruik van mechanische ventilatie tijdens rustperioden, zoals onder de milde weersomstandigheden tijdens dit onderzoek.

Summary

Consumer concerns about animal welfare during transportation of animals across Europe continue to grow. European legislation is aimed at improving regulation of transportation of live animals. Basically, present legislature states that live animals should be able to move, stand and lay down freely within their thermal comfort zone without risk of injury, discomfort or suffering. At the request of EL&I research was performed into transportation of pigs over long journeys (> 8 hours). This study comprised eight road journeys performed at one of two stocking densities with slaughter pigs. The pigs were transported in the period between June and October 2010 from a farm in Schleswig-Holstein across Germany to a slaughterhouse in district Dahme-Spreewald in the state of Brandenburg in Eastern Germany. The conditions under which the pigs were transported received particular attention with regard to animal welfare. Several factors that influence the well-being of animals were registered including: climatic conditions (temperature, wind speed, humidity) or vehicle associated factors (i.e. vibration, acceleration). Ventilation, space allowance and standing room, or in other words the amount of free space around and above the animals, are important factors influencing their living climate during transportation (Van Reenen et al, 2008). Pigs are considered to be highly sensitive to travelling conditions yet capable of adapting to new situations. Certain physiological indicators such as blood levels of cortisol, total protein, albumin, CK, white blood cell counts, Ht and Hb were measured prior to departure from the farm and after arrival at the slaughterhouse.
Other physiological indicators such as body temperature (ibutton thermo-logger) and heart rate (telemetric logging device) were monitored throughout each journey in 8 test animals placed in four compartments to the front and rear of the uppermost and lower deck of the vehicle. Behaviour of the pigs was monitored via video cameras placed in the front and rear compartments of the upper and lower deck. Duration of transportation and placement of the animals in the vehicle have been known to influence animal behaviour (Averios et al 2007, Warriss 1998). Placement in the vehicle has been suggested to contribute to stress and mortalities, often in relation to high stocking densities.
Therefore, the immediate aim of this study was to address the conditions of transportation and determine whether or not animals transported at a space allowance below the legally required 235 kg /m^2 could adapt adequately. Assessment of the adaptability of the transported pigs was based on the results of analyses of the above mentioned parameters performed during 8 journeys under commercial transport conditions.
Results indicate that during loading body temperature of the pigs increased more in the normal density groups compared to the low density groups. Since all groups of animals are treated equally during the experiments the increase during loading appears to be related to higher activity or to stress associated with less space in the transport vehicle. During transport the body temperature decreased consistently within 1 hour to normal baseline values but, at the higher loading densities body temperature remained slightly higher than at low loading densities
The same trend is observed in heart rate as in body temperature. Hart rate is considerably increased compared to normal baseline values during the loading period. As soon as the animals are all loaded and the vehicle is closed heart rate starts to decline consistently to normal baseline values. Although not significant, heart rate remains higher in the normal loading density groups compared to the low density groups. Remarkable observation is the increase in heart rate during the lunch break of the driver. Short after parking the transport lorry heart rate starts to incline however, the incline is more vigorous for pigs at higher loading densities. After a short period body temperature of all pigs starts to follow the heart rate and inclines as well during the break. The increase in heart rate and body temperature is not as suspected when looking at the resting behaviour of the pigs. Since more than 80% of the pigs is laying down during the driving brake it would expected that body temperature and heart rate remained at normal levels. When looking at the environmental conditions at driving and resting it is striking that there is no ventilation during the lunch break and as a result ambient temperature in the lorry is increasing. It can be assumed that due to the increased ambient temperature pigs need higher circulation levels i.e., higher heartbeat, to keep the body temperature constant.
Signs of fatigue can be observed in changes in muscular enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH). Changes in aspartate aminotransferase (AST) during transportation have in the past been seen to indicate muscular membrane degeneration resulting from road transportation. Low loading densities resulted in a close to significant lower level of CK and AST in the blood compared to the normal loading density. Taking into account the longer duration of fighting and the shorter period of resting in the normal density it is reasonable that pigs transported at higher loading densities are more fatigued than animals transported at lower densities.

Looking back at cortisol levels that are higher at normal densities and comparing them with the CK and AST levels and the shorter resting periods it can be argued that it is conceivable that pigs at normal density are more stressed than at low loading densities.
In conclusion, there are differences observed between journeys and between loading densities but due to the large differences between individual transports, a sustainable conclusion on animal welfare in favour of normal or low loading density is not possible.
However, the differences in responses of pigs during transport (i.e. fighting and resting behaviour), in heart rate levels and in blood values tend to indicate that pigs with more living space are more capable of adapting to transport conditions.
These conclusions are based on the following;

- Incidence of fighting bouts were influenced by loading density, period within the transport and formation of travelling groups by mixing unfamiliar animals.
- Heart rate and body temperature are increased during handling and loading clearly indicating that this is a stressful part of the total transport.
- The differences in cortisol, creatine kinase and aspartate aminotransferase are however not significantly, higher at normal loading density compared to the lower loading density which is indicative for fatigue due to more activity or less resting and more fighting at higher loading densities.
- At lower loading densities a large number of pigs are already laying down during travelling while at higher loading densities almost no animals are laying down during travelling therefore, It can be that concluded if pigs have enough space they will lay down for resting during driving and already within 3-4 hours after departure.
- Water usage per animal was similar irrespective loading density. During transport pigs use the drinkers and are willing to drink at the start of the journey.
- Results from this study indicate a trend towards overriding physiological and ethological thresholds caused by stress as pigs attempt to adapt to transportation over long distances. This can only be addressed with limited management tools, such as ventilation enhancement during breaks particularly during moderate weather conditions such as those experienced during this study.

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1 Introduction

There is an increasing demand from consumers and public organisations alike for improvements in the rearing and handling of animals. Particularly those animals involved in the food production chain. Concerns regarding the impact of transportation on animal welfare are on the increase (Mota-Rojas et al, 2006; Becerill-Herrera et al, 2007). Parliamentarians, policy makers and various governmental organisations are aware of the fact that something has to be done to address these public concerns. Animals are transported for various reasons whether it be pedigree stock transferred between farms or slaughter animals from farm to slaughter facility. This is for the animals in question a stressful procedure and often the cause of significant losses (Grandin, 2000, Werner & Gallo, 2008, Carter & Gallo, 2008). Therefore the conditions under which animals are transported require particular attention if we are to secure a minimum risk to animal welfare. During such journeys there are several factors that influence the well-being of animals which may be influenced by the climate (extremes of temperature) or vehicle associated (vibration, acceleration, driving skills, noise, space), even animal related factors (including mixing, hunger, thirst, fatigue). Their welfare maybe compromised by any or all of these factors in relation to the length of the journey involved. In principal, European legislature is aimed at improving the regulation of conditions of transportation for all animals. The basic principle that animals during transport should have sufficient room to stand and lay down freely in a natural position within their thermal comfort zone without risk of injury or suffering has been laid down in EU legislature. The EU transport decree 1/2005 demanded sufficient laying space for animals and adequate ventilation. Ventilation, space allowance and standing room, or otherwise the amount of free air space above the animals, are important factors influencing the living climate during transportation. However, until now although there has been some research into the various aspects of animal transportation there has been insufficient coordination of the information that has become available from research. This lack of cohesion has hindered any comprehensive determination of the potential influence of the abovementioned factors on animal welfare during transportation.
In addition to measurements of conditions during transportation (e.g. temperature, humidity, precipitation) or driving conditions (e.g. velocity, duration, acceleration, driving style) the animal itself also displays a physiological and ethological response to the conditions under which it is transported. Pigs are generally considered to be highly sensitive to travelling conditions but are capable of adapting to new situations (Becerril-Herrera et al, 2010). These responses include indicators of physiological stress such as increased levels of cortisol with corresponding reductions in ascorbic acid as discussed by Warriss, 1998. Incidences of travel sickness in pigs at various levels of severity have also been acknowledged. Weight loss has also been advocated by several authors as an indicator of compromised conditions of transport often in relation to dehydration, which is subsequently displayed in higher levels of total protein in plasma samples taken at slaughter (Warriss, 1998).

Not only the journey itself is stressful to the animal but loading and unloading are regarded as challenging events during the transportation procedure. During loading and unloading it has been recorded (van Putten & Elshof, 1978) that heart rate increases and often intensifies as the handlers' methods of encouragement become more robust. Certain strains of pig have been identified to be more sensitive to stress and some animals do die during transport. Mortality during transport also reflects the conditions of welfare. It has been shown that a seasonal relationship exists between ambient temperature and pig welfare during transport (Lendfers 1970; Allen & Smith, 1974; Warriss & Brown, 1994). Pigs are considered to be particularly sensitive to extremely high temperatures; they are incapable of transpiring and react by panting in an attempt to adjust their respiration rate in order to cope with their situation. Concerns have been documented on the ability of slaughter pigs to effectively regulate their body temperature during transport (Warriss et al 2006) Additional complications arising from high humidity values have been recognized but it remains uncertain what the effect of humidity is on pig welfare.
Placement in the vehicle has been suggested to contribute to mortalities. Earlier work (Sains 1980; Riches et al., 1996) has shown that marginally more pigs die on the bottom deck in both winter and summer. Sains (1980) also found that most deaths occur in the compartments immediately behind the cab, possibly due to inferior ventilation in this part of the vehicle. Studies performed in the UK (Meat and Livestock commission, 1993; Warriss et al 2006) have shown that animals travelling in the front of the vehicle displayed higher cortisol, albumin, osmolality and CK levels than their counterparts in other compartments of the same vehicle.
It has been shown that higher stocking density during transport increases the risk of mortality. In earlier studies (Lendfers, 1971) more deaths were registered in pigs transported at densities above 1.2 animals per m^2. Others have recorded a higher incidence of deaths (0.54%) in groups of pigs

carried at recommended densities or above, decreasing progressively as density decreased to 80 % of the recommended level. A survey of commercial practice in the UK (Riches et al., 1996) also showed higher incidences of death in batches of pigs transported at 239 kg per m^2 (0.42 m^2 per 100kg).
There is evidence to suggest that distance travelled may also affect animal welfare (Averos et al, 2007). In journeys ranging between 150 to 530 km mortality was seen to increase progressively from 0.21 to 0.65%.
Yet others found no effect from length of journey (reviewed by Warriss, 1998). These contradictory findings may be explained by an interaction between ambient temperature and journey length: at temperatures below 10^{o}C, distances from 5 to 45 km had no effect while at temperatures of 10 to 15^{o}C or above deaths became more frequent on longer journeys (Lendfers, 1971).

The immediate aim of this study was to address the conditions of transportation and determine whether or not animals transported at a space allowance below the legally required 235 kg /m^2 could adapt adequately. Assessment of the adaptability of the transported pigs was based on analysis of measurements performed during 8 journeys under commercial transport conditions across Germany in the summer and autumn of 2010.
It is intended that findings from this research will contribute to the improvement of transportation conditions of slaughter animals and pigs in particular. In practice, it is has been seen that adjustments to (EU) legislature on transportation is difficult and only has a chance of succeeding when substantiated with evidence from scientific research.

2 Materials and Methods

Contact was sought with a commercial transport organization in district Schleswig-Flensburg (state of Schleswig-Holstein, Northern Germany) for the transportation of fattening pigs from Schnarup-Thumby to the VION slaughter facilities situated in Kasel-Golzig in district Dahme-Spreewald (state of Brandenburg, Eastern Germany). This was an average distance by road of approximately 550km. Eight journeys (hereafter referred to as trips) were followed in the period from June – October 2010.

Figure 1: Transport vehicle

Prior to performance of these journeys approval was obtained from the Wageningen UR Livestock Research ethical committee for the performance of animal experiments.

2.1 Experimental design and procedure

The fattening pigs (average bwt 110 kg) were provided by a commercial pig farmer and loaded at their production farm in Schnarup Thumby. The pigs were transported in one of two treatment groups based on space allowance. These treatment density groups were targeted at a standard space allowance of 235 kg per m^2 (referred to as normal density indicated as ND) or a lower alternative density of 185 kg per m^2 (referred to as low density indicated as LD).
The animals were transported in a three tiered vehicle (Figure 1) of which the front and rear compartments of the upper and lower decks were designated as experimental units (Figure 2, indicated with blue spots). Each trip was performed at one of the two space allowances with two trips being performed within the same week (Table 1). In total 8 trips were performed in the period June to October 2010. Pigs were also transported in all other compartments during each trip in order to complete the commercial load, but those animals were not monitored individually.

Each pig experienced a trip composed of five phases referred to hereafter as legs.

- The first leg began as the pig enters the vehicle. This leg also contained a short journey from the pig farm to the weighbridge.
- The second leg began with departure from the farm and comprised a large part of the transport in the direction of Berlin.
- Leg 3 was a compulsory pause for the driver (46 ± 2 minutes) in the vicinity of Berlin.
- The fourth leg of each trip was a shorter drive (approximately 2 hours) to the slaughterhouse in Kasel-Golzig.
- The final phase (leg 5) comprised the period taken to enter the slaughter plant, weigh the vehicle and ends as the pig leaves the vehicle for placement in lairage.

However, it was realised that the waiting times varied considerably between individual pigs initially due to blood sampling and preparation and placement of logging devices and finally due to time taken to take a blood sample and disconnect and remove logging devices in slaughterhouse lairage. Therefore, the period of measurement varies per individual depending on the type of measurement. Measurement period relevant to each parameter is given in the results.

Prior to each journey 12 (twelve) females were assigned as registration animals. They were used for measuring heart activity, body temperature, and to provide blood samples prior to and after the journey. Eight of these pigs were used for continual heart activity (ECG) registration and 4 were used for continual heart beat (bpm) registration. All registration animals were allocated to one of the four experimental units on the upper or lower deck, i.e. 3 registration animals per compartment of which 2 animals with ECG devices.

The pigs were weighed and sorted into groups by the farm personnel. All animals were fasted for 8 hours prior to transit. Loading sequence was from front top to lower rear. Upon arrival at the slaughterhouse the registration animals were placed in a reception pen and blood samples were taken immediately. Heart activity loggers and body temperature loggers were deactivated and removed approximately one hour after unloading at the slaughterhouse. Unloading sequence was lower rear to front top.

2.2 Measurements

2.2.1 Environmental factors

To measure the external conditions during each journey the transport truck was provided with sensors. The positioning of the sensors is indicated in Figure 2.

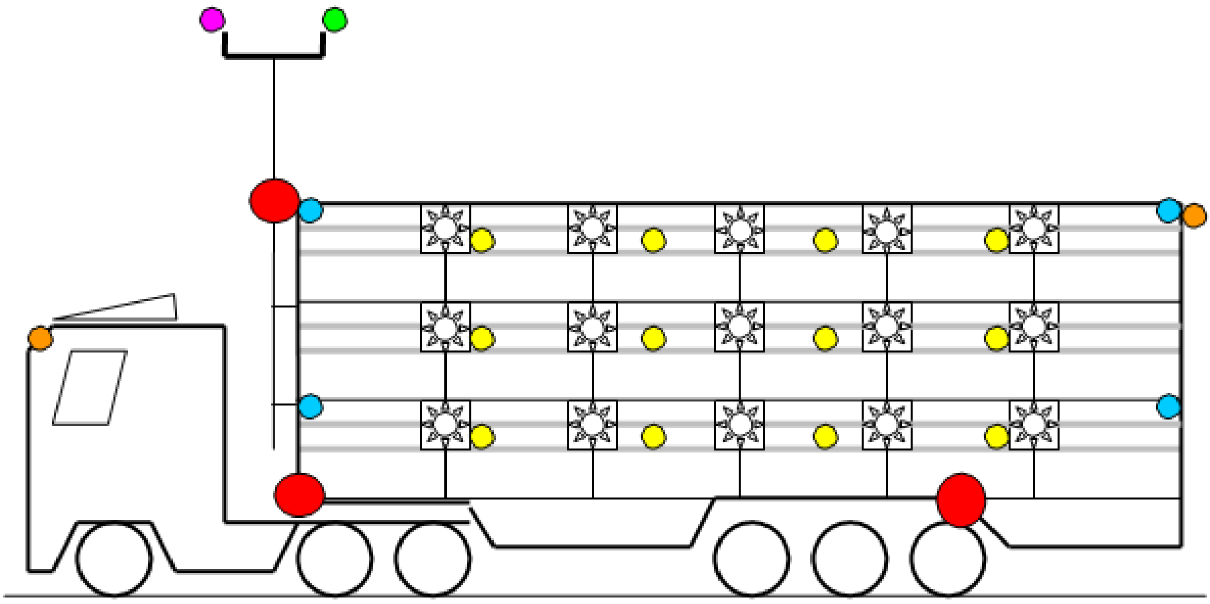

Figure 2: Placement of sensors and cameras in vehicle. Different coloured spots indicate placement of sensors for three dimensional movements of vehicle (red); sensors for outside temperature and humidity (orange), inside temperature, humidity and air velocity (yellow), positioning of cameras (blue), measurement of wind speed (green) and wind direction (pink).

3D-acceleration was measured every second by three 3-dimensional movement sensors and registered as 10 second averages. The meteorological measurements were performed and registered every minute. Parameters measured during each journey included temperature (in- and outdoor), humidity (in- and outdoor), wind speed (outdoor), air velocity (indoor), ventilation fans (on/off) on each deck, and truck speed.

Wind speed and wind direction (every minute) were measured occasionally when the vane could be extended, i.e. prior to departure, during lengthy pauses and upon arrival. Water usage was measured in litres per minute, with the whole vehicle as unit of measurement.

All data was recorded on a 64 channel data logger (combination of e.bloxx and e.reader, Gantner Instruments GmbH, Schruns, Austria) which was placed in one of the storage compartments of the vehicle.

2.2.2 Behaviour

Animal behaviour was recorded using 4 wide lens video cameras placed at the front and rear of the upper and lower decks (blue spots, Figure 2). The camera type was Santec VTC-E100P colour corner mini camera, 550 TVL, 2.3 mm lens, 12 V DC (Sanyo Video Vertrieb AG, Ahrensburg, Germany). Each camera was placed at the rear or front of the upper or lower deck, so as to cover as much of the area of the experimental compartment as possible.

Behavioural observations were performed for all animals in the experimental compartments, i.e. upper front, upper rear, lower front, lower rear compartments. Video analysis was performed from closure of the experimental compartment at loading (and floor having been raised, for upper compartments) until opening of the compartment at unloading (or floor being lowered, for upper compartments). Video files were examined for the following behaviours:

- Activity: activity was measured at 10 minute intervals as the number of active or moving animals observed during a period of 2 minutes
- Posture: lying down, standing and sitting were observed in each study compartment at 5 min intervals
- Fighting: frequency and duration of fighting (aggression involving one or more pigs) was observed by continuous analysis of full video files of the 4 study compartments

2.2.3 Heart beat and body temperature

Prior to each journey eight registration animals were fitted with remote stand-alone Telemetric data loggers (Lowe et al, 2007) for tracing heart activity (ECG) throughout each journey. Each monitored pig was fitted with a data logger for continual heart beat registration. Pad electrodes (Unilect™ 5cm diameter, Unomedical Ltd., Stonehouse, GB) were fitted to the thorax immediately after shaving the area and cleansing with 70% alcohol. An elasticated belt was attached to protect the leads attached to the electrodes that were joined to the data logger placed in a small metal box which was housed in a leather pouch on the back of the pig (Figure 3 to Figure 6).

Figure 4: Elasticated belt containing logging equipment

Figure 4: Ppositioning of pad electrodes and leads protected with surgical tape

Figure 6: Placement of belt

Figure 6: Pig equipped with logger in lairage prior to departure

Three pad electrodes (positive, negative and neutral) were fixed to the skin with surgical glue to ensure a good contact immediately behind the elbow on each side of the pigs' breast. The belt was tightened and both belt and pouch were held in position using heavy duty duct tape (Figure 6). Data logging began immediately after securing the belt and pouch and the registration pigs were held in a separate pen prior to loading. During trip numbers 5 to 8 an additional protective cover was placed over the pouch and belt which also meant that duct tape was no longer required to secure the belt (Figure 7).

Figure 8: Pigs with extra protective cover in lairage pen prior to loading

Figure 8: Pigs loaded in vehicle compartment prior to departure

The other 4 registration animals were equipped with Polar® devices (Polar Electro Oy, Kempele, Finland) for registration of heart beat (beats per minute). The equipment consisted of an electrode belt with build-in transmitter (T31) and a wristwatch receiver (Accurex Plus^{TM}), see Figure 9. Electrode gel (Signa Gel®, Parker laboratories Inc., Fairfield, New Jersey) was used to optimize contact between electrodes and skin. Data from the transmitter was telemetrically transferred to the receiver which was set to store at 1 minute intervals. The receiver was placed in a protective box and attached to the electrode belt, where after the equipment was further protected by a 30 cm wide elastic rubber belt (Figure 10).

Figure 10: Polar® receiver, electrodes with transmitter (both sides)

Figure 10: Animal equipped with Polar® device and protective belt

Body (internal) temperature in the 12 registration animals was measured continually during each journey journey using iButton® Devices, type DS1921H thermo sensors (Maxim Dallas, TX, USA (www.maxim-(www.maxim-ic.com) set to store at 1 minute intervals. Sensors were connected to a rubber ring by heat heat shrink and inserted into the vagina by modified 20 ml syringe (

Figure 11). After placement, the unfolding force of the rubber ring ideally held the iButton in place.

Figure 11: Syringe, rubber ring, iButton and heat shrink, used to measure body temperature

Upon arrival at the slaughterhouse all 12 monitor pigs were separated into a quarantine pen (

Figure 12) during unloading and blood samples taken immediately after completion of the unloading procedure. The thermo-sensors were also recovered from the vagina. All loggers that were still active were deactivated and removed approximately one hour after unloading.

Figure 12: Logger control in lairage at slaughterhouse

Heart activity (ECG) was registered and analysed as average heart beat per min for each 5 minute period (Labchart7 Pro, V7.1.2, AD Instruments, Cologne, Germany). Analysis of cardiac function was based further on an assessment of heart rate variability (HRV) which has been advocated in several studies reviewed by Von Borell et al., 2007. Computation and analysis of HRV data was performed using Kubios software (Kubios HRV, version 2.0. Tarvainen en Niskanen, 2008).
Fast Fourier transformation (FFT) was used to decipher and analyse the heart beat signals obtained from the telemetric logging devices. According to studies reviewed by Von Borell et al., 2007, several studies have recommended analysis in sections of at least 512 points. Our data was analysed using KUBIOS software set at 512 points allowing a 25% overlap in frequency bands. Wave frequency bands were set at <4 for very low frequency (VLF), 0.04 – 0.13 for low frequency (LF) and 0.13 – 0.4 for high frequencies (HF) in accordance with recommendations for swine reported in a recent review (Von Borell et al., 2007). It is considered that when assessing HRV, time domain measurements reflect various aspects that indicate import differences in inter beat interval (IBI) data series. A parameter such as RMSSD (determined as the square root of the average of the sum of the square roots of differences between consecutive IBIs) is particularly interesting in the estimation of high frequency inter beat variation representing vagal regulatory activity. One of the benefits of FFT analysis is then in the ability to assign underlying physiological heart functions to wave frequency bands e.g. HF as indicator of vagal activity and VLF as indicator for sympathetic or sympathetic combined with vagal activity. Therefore, the LF: HF ratio was also seen as an important measure of the sympatho-vagal balance. Furthermore, to account for differences between individual animals LF and HF power can be expressed on a normalised scale whereby the absolute power is calculated as the ratio of LF: total power.
Heart rate data from the telemetric logger were analysed according to the settings mentioned above for each leg of the journey and for journeys as a whole. As long periods of measurement could possibly level out differences in HRV, these parameters were also calculated for multiple 5-minute segments which were distributed across the legs and always involving the 5-min periods at the beginning and end of each leg of the journey. Twenty-seven such 5-min segments were identified: the numbers per legs 1, 2, 3, 4 and 5 are 3, 11, 5, 6 and 2 segments respectively. Time domain measures that are included in the analyses are SDRR (standard deviation of all inter-beat intervals, unit ms) and RMSSD (root mean square of differences between successive inter-beat intervals, unit ms). Low frequency is presented in proportion to HF (LF/HF) and HF as a percentage of total power (HF/Total Power x 100).

2.2.4 *Blood parameters*

It has been widely indicated in earlier work (Averós et al, 2009, Calà et al., 2009, Knowles and Warriss, 2007), that stress during transportation can cause an increase in blood levels of cortisol (during short journeys; Averós et al, 2007) (Cort) and glucose (Glu) concentrations and changes in white blood cell (WBC) counts. Muscular fatigue can be observed in changes in muscular enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH). Changes in aspartate aminotransferase (AST) during transportation have in the past been seen to indicate muscular membrane degeneration resulting from road transportation (Adenkola et al, 2009). Increases in blood albumin and total protein concentrations can indicate an onset of the dehydration process (Averós et al, 2009, Averós et al., 2007) in the body along with haematocrit (Ht) and red blood cell (RBC) counts.

To this end two samples (9 ml) were taken from the neck (Jugular vein,

Figure 13) in EDTA-K coated S-monovette® tubes (Sarstedt AG& CO, Nümbrecht, Germany). The first sample was taken before transport and prior to any other handling or treatment; the second sample was taken immediately after arrival at the slaughterhouse in the reception pen. Animals were restrained by snout snare to facilitate sampling. Samples were stored on ice until further processing after all samples had been collected.

Figure 13: Blood sampled from the neck

After blood collection, two micro haematocrit capillary tubes (Brand, Werthheim, Germany) per sample were filled with blood, sealed with wax, and centrifuged at 13000 rpm for 5 minutes to assess haematocrit (Ht) levels in blood. Plates of blood smear were prepared and fixated in methanol (5 minutes) until they were dry and transported to lab. The remaining part of the blood samples was centrifuged at 4000 rpm for 10 minutes, and aliquots of plasma were kept on ice until the last sample

was processed. Plasma samples were then frozen in liquid nitrogen, transported to lab, and stored at -80 °C until required for analysis.
Blood smear plates were stained using the Pappenheim (May-Grünwald-Giemsa) method at the FLI, Celle. Counts of the first 100 white blood cell components (leucocytes) were made under a microscope and identified as lymphocytes, neutrophil, basophil, eosinophil or monocytes. Plasma samples were analysed by a COBAS® Mira S analyser (Hoffmann-La Roche, Basel, Switzerland) in the Clinic for Swine and Small Ruminants (University of Veterinary Medicine, Hannover, Germany) for levels of protein (Pro), albumin (Lab), ratio of Globulin to Albumin (Global), glucose (Glu), L-lactate (Lac), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and creatine kinase (Ck). Cortisol (Cort) in plasma was analysed using ELISA kits (Cortisol ELISA, RE52611 and RE52061, IBL International GmbH, Hamburg, Germany) at the FLI, Celle, Germany.

2.3 Statistical analysis

Data obtained from the various aspects of the study including environmental conditions or animal related measurements such as heart rate, HRV, body temperature, blood parameters and behavioural characteristics were analysed using interactive software (GenStat, 2010). Initial screening of the data based on a student's T-test was followed by REML variance component analysis (André et al, 2011) using the general model for analysis of heart rate data, HRV, and body temperature and certain aspects of behaviour (i.e. movement and fighting):

Response variate: y
Fixed model: Constant + Leg + Density + FR + LT + Leg.Density + Leg.FR + Density.FR + Leg.LT + Density.LT + FR.LT + Leg.Density.FR + Leg.Density.LT + Leg.FR.LT + Density.FR.LT
Random model: Trip + Trip.Comp + Trip.Comp.Leg

Where: leg = phase of journey (n=1-5), density = space allowance (normal or low), F=front compartments, R=rear compartments, T = upper deck, L = lower deck.

For analysis of the blood parameters the initial measurement was included into the above model as covariate, this in an attempt to avoid any systematic distortion due to sampling or other causes. Where required (i.e. fighting, LDH, AST CK and L-lactate) a log transformation of the input data was performed to facilitate a correct analysis.

Data from the white blood cell counts were analysed using a generalized linear mixed model:

Method: c.f. Schall (1991) Biometrika
Response variate: monocyten
Distribution: poisson
Link function: logarithm
Random model: Trip
Fixed model: Constant + covar + Density + FR + LT + (Density.FR) + (Density.LT) + (FR.LT) + (Density.FR.LT)

When analyzing the log of the count, + 0.01 was used as covariate.

3 Results

3.1 Journeys

Eight journeys were made in the period from 21st June to 13th October 2010. Each trip covered the same route of approximately 550 km, of which 460 km was motorway. Road-, traffic- and weather conditions differed between trips. Average timing for the different legs is presented in Figure 14.

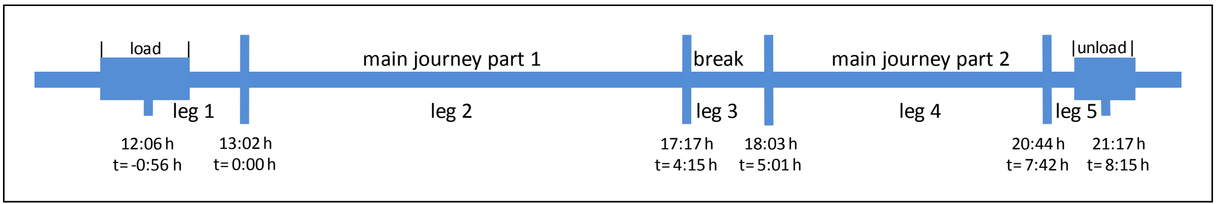

Figure 14: Average timing for each leg of the trips with slaughter pigs

Loading of the pigs commenced at 11:34 ± 0:42h and the loading process took 59 ± 8 minutes. Unloading took 31 ± 4 minutes and was completed by 21:33 ± 1:04h. Details and timing of individual trips are presented in Table 1.

The average loading on 'normal' density trips was 233 kg/m^2 and the average loading on 'low' density trips was 179 kg/m^2 (i.e. 77% of 'normal' density). On 'normal' density trips the compartments on the middle and lower decks always held 13 pigs, whereas the upper deck compartments held 12 pigs, except for trip 8 when also the upper compartments held 13 pigs because of lower body weights. Therefore, the calculated loading density in study-compartments on 'normal' density trips was slightly below truck average, i.e. 230 kg/m^2 . On 'low' density trips all compartments on all decks always held 10 pigs, and therefore calculated loading density in 'low' density study-compartments was equal to truck average, that is 179 kg/m^2 (78% of 'normal' density study compartments).

Table 1: Details of trips with slaughter pigs

Trip	Date	Density	Number of pigs loaded	Avg body weight (kg)	Actual density (kg/m^2)	Duration of leg 1 (h:mm)	Duration of leg 2 (h:mm)	Duration of leg 3 (h:mm)	Duration of leg 4 (h:mm)	Duration of leg 5 (h:mm)	Load to unload, legs 1..5 (hh:mm)
1	21-06-10	normal	190	115	226	1:04	4:10	0:48	2:34	0:35	9:11
2	23-06-10	low	150	111	172	0:58	4:07	0:49	2:50	0:23	9:08
3	13-09-10	normal	190	120	237	1:21	4:14	0:45	2:41	0:43	9:45
4	15-09-10	low	150	111	172	0:41	4:09	0:44	2:33	0:33	8:41
5	27-09-10	normal	190	123	242	1:01	4:15	0:45	3:26	0:40	10:07
6	29-09-10	low	150	121	188	0:44	4:20	0:50	2:17	0:34	8:45
7	11-10-10	low	150	118	184	0:59	4:24	0:45	2:30	0:27	9:05
8	13-10-10	normal	195	111	225	0:44	4:16	0:45	2:35	0:25	8:45
Avg.				116	206	0:56	4:14	0:46	2:40	0:32	9:11
Std.				4.9	29.6	0:13	0:05	0:02	0:20	0:07	0:30

Leg1: from loading until start of main journey
Leg 2: first part of main journey
Leg 3: halfway break
Leg 4: second part of main journey
Leg 5: from end of main journey until unloading

3.2 Environmental factors

Trips 1 and 2 were carried out on days with warm summer weather and many hours of daylight. Trips 3 and 4 were performed in late summer weather, with occasional gusts of wind during trip 4. Trip 5 experienced heavy rainfall and a one hour delay due to traffic congestion around Berlin. Light rainfall occurred during trips 6 and 7. During trips 7 and 8 the evenings were cold and there were fewer hours of daylight.

3.2.1 Truck movement

All journeys were executed within a normal range of driving; no accidents occurred or emergency manoeuvres were required. Speed of driving was up to 90 km/h on motorways. Figure 15 shows a typical example of the speed of the truck during trip 7. Departure was at 12:36h with a break at 17:00h and arrival at 20:15h.

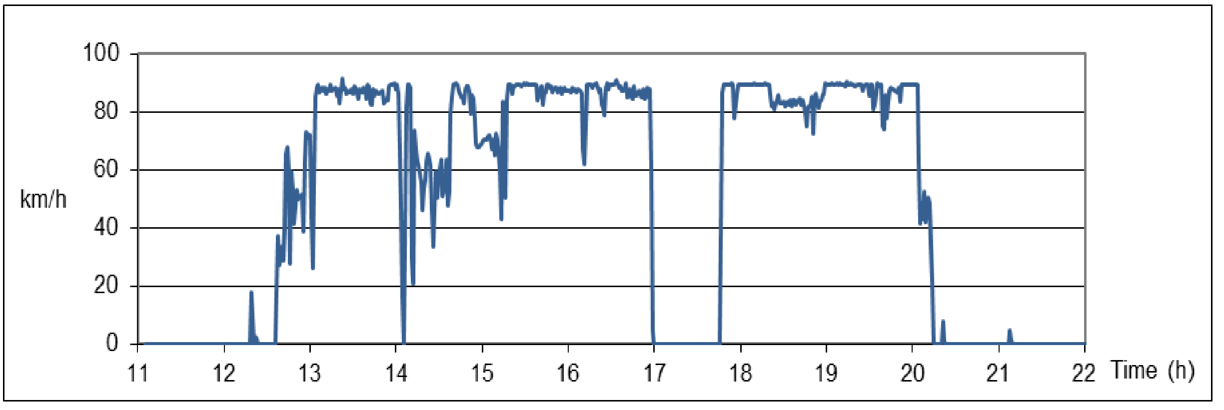

Figure 15: Typical example of driving speed during one of the trips

Vibration of the truck was measured by 3-dimensional accelerometers placed at 3 locations on the outside of the truck. During trips 1, 2 and 8 one of these sensors was defect. No peculiarities of acceleration were recorded. We found differences in the level of acceleration between sensors at different locations and differences in the directions of acceleration. Overall in the period between departure from loading platform and arrival at the unloading bay, the front-top sensor showed 2.5 times more vibration, and the rear-low sensor showed 1.3 times more vibration than the front-low sensor. This pattern was seen on all trips, and indicates that the front-upper deck vibrates twice as much as the lower decks irrespective of loading density. No differences were observed between ND and LD trips in terms of direction of vibrations. Accelerations in the front-rear direction were lower than in other directions. Left-right movement was 1.8 times greater, and up-down movement was 2.0 times greater than front-rear movement, indicating that most of the movement that pigs experienced in transit was in the up and down direction. This was most pronounced in the front-top and rear-low location of sensors.

3.2.2 Outdoor climate

Relative humidity and temperature were measured by two sensors on the outside of the truck. The sensor located on the upper-front was defect on trips 1, 3, 4. Figure 16 shows the averaged patterns during the journeys, synchronized for time of departure (start of leg 2 at t=0h). The average values per trip are presented in Table 2.

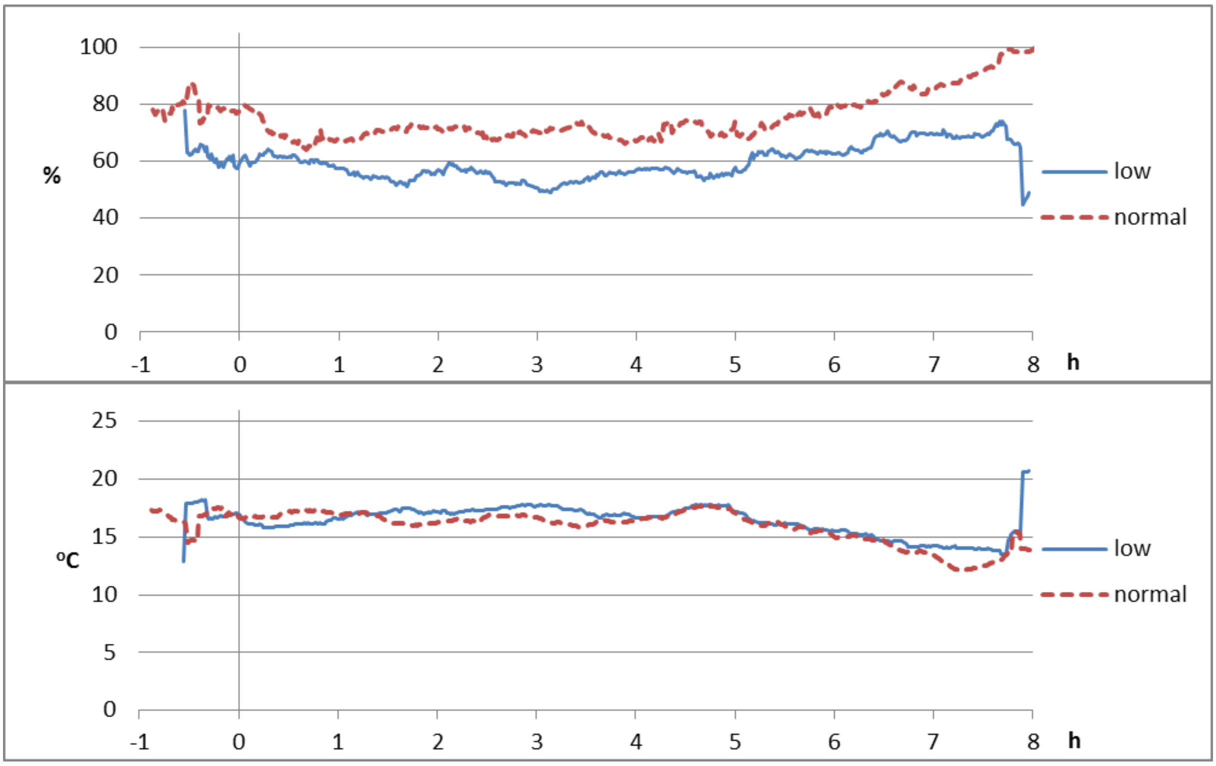

Figure 16: Average pattern of outside relative humidity and temperature during trips at low and normal density (departure time 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

Average outdoor temperatures showed quite similar patterns, and levels did not differ between LD and ND journeys. Trips 1 and 2 were on warm days (average >20^{o} C). On average outdoor relative humidity was approximately 15% higher during ND trips. However, this was influenced by the very rainy conditions experienced during trip 5. Abrupt changes in pattern around 7:45h after departure are due to the fact that most trips had been completed by this time and recordings ceased, leaving a single trip to make up the average.

Wind speed was occasionally measured during stationary periods but did not show extreme values or relevant differences.

Table 2: Trip averages for outside relative humidity and temperature

Density	Trip	RH (%)	Temp (°C)
low	2	42	22.9
	4	60	17.8
	6	66	12.5
	7	70	12.8
Avg. low		**60**	**16.4**
normal	1	54	20.7
	3	81	17.6
	5	94	14.0
	8	63	14.6
Avg. normal		**76**	**15.9**

3.2.3 *Indoor climate*

Climate inside the vehicle was measured by sensors (30 in all) mounted on gates between the compartments on all decks. Defective sensors were observed on twelve occasions; these failures were randomly distributed throughout the vehicle and trips. Figure 17 shows the averaged patterns during the journeys, synchronized for time of departure (start of leg 2 at t=0h). The average values per trip are presented in Table 3.

Indoor air speed, relative humidity and temperature show comparable patterns throughout the day between ND and LD trips. Air speed also shows a direct link to driving speed, clearly indicating driver's break approx. between 4:15 to 5:00h after departure. During this break, relative humidity and temperature appear to increase, which is most distinct to ND trips. Towards the evening, relative humidity rises, and temperature decreases. Abrupt pattern changes around 7:45h after departure are due to the fact that most trips had been completed by this time and recordings ceased, leaving a single trip to make up the average.

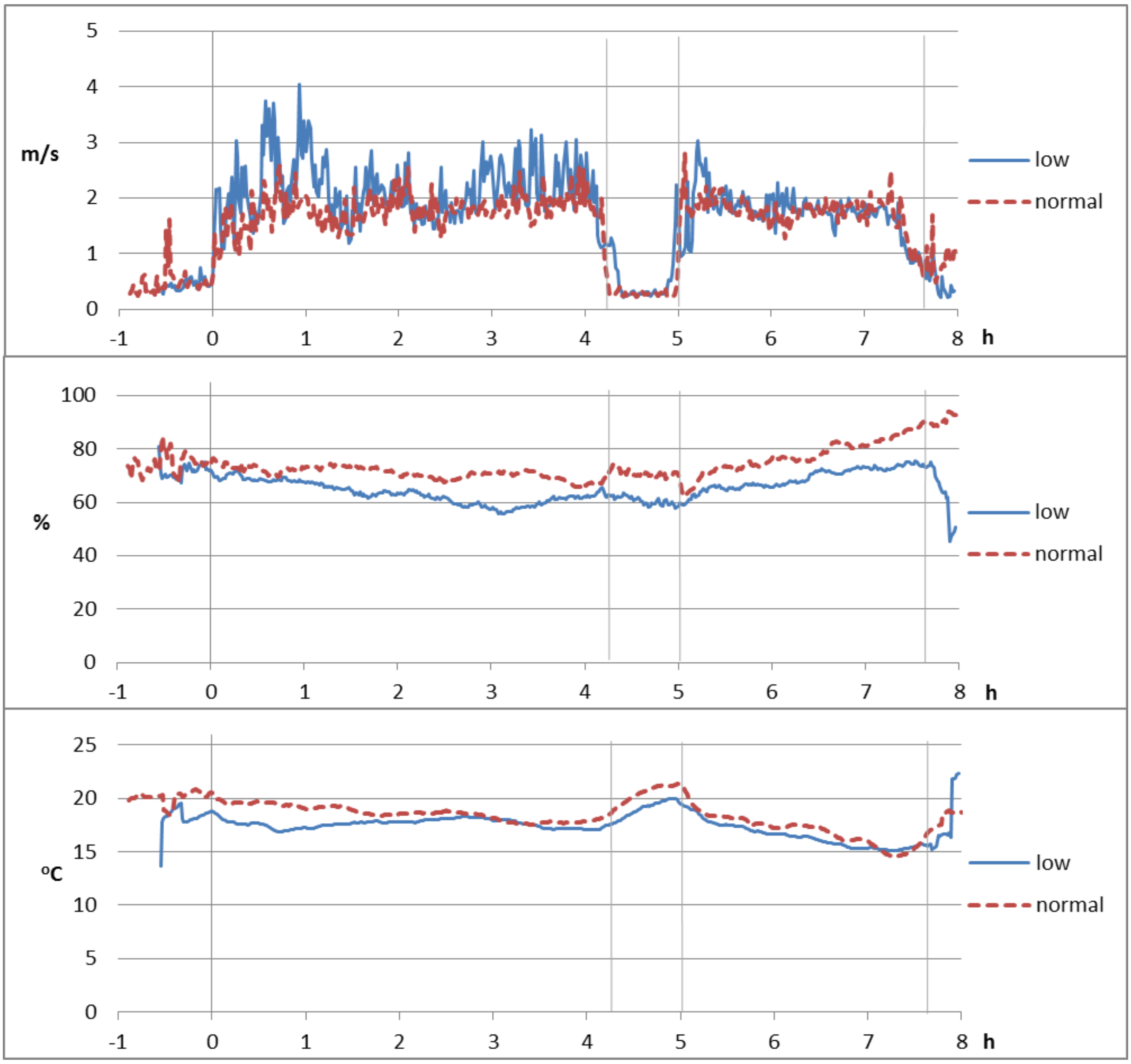

Figure 17 : Average patterns of air speed (m/s), relative humidity (%) and temperature (°C) measured inside the vehicle during trips at low and normal density (departure time 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

Table 3: Trip averages for air speed, relative humidity and temperature inside the vehicle

Density	Trip	Airspeed (m/s)	RH (%)	Temp (^oC)
low	2	1.80	46	23.4
	4	2.29	70	17.6
	6	1.43	72	14.6
	7	1.53	73	14.5
Avg. low		**1.74**	**65**	**17.4**
normal	1	1.54	54	21.0
	3	1.39	76	19.9
	5	1.66	89	16.8
	8	1.42	73	16.2
Avg. normal		**1.51**	**74**	**18.3**

Average air speed inside the vehicle was slightly higher in LD trips, due mainly to trip 4. Humidity was highest on ND trips throughout the day; the wet conditions experienced during trip 5 were particularly influential to this average. Average indoor temperatures were slightly higher on ND trips throughout most of the day.

Independent of loading density, air speed in the vehicle decreased from 2.04 m/s (front compartments) to 1.26 m/s (rear compartments), whereas humidity and temperature displayed no such decline. Air speed on the middle deck was lower (1.42 m/s) than on the upper (1.66 m/s) and lower decks (1.79 m/s). Most extreme differences in air speed were observed between lower front (2.3 m/s, highest) and lower rear (1.1 m/s, lowest) compartment. Humidity remained within a similar range on all decks and compartments. Temperature on the top deck was lower (17.2 ^oC) than on the middle (18.2 ^oC) and lower decks (18.4 ^oC). Greatest differences were observed between lower front (19.3 ^oC, highest) and upper front (16.6 ^oC, lowest) compartments. The lower front compartment appeared to display the highest temperature despite experiencing the highest wind speeds.

The automatic ventilators on each deck were rarely activated. During trips 1 and 2 the ventilators were only active in the period after each deck had been fully loaded until departure from the barn; on trip 2 ventilators were active on each deck in the period from parking at the unloading bay until actual opening of each deck. Trips 1 and 2 occurred on warm summer days (>20^oC), of which trip 2 was warmest. Other to these incidences the ventilators were never active.

3.3 Behaviour

Behaviour was registered by a camera in each of the four study compartments throughout the journey and recorded on digital video files. Video analysis was performed from closure of the study compartment at loading (and floor having been raised, for upper compartments) until opening of the compartment at unloading (or floor being lowered, for upper compartments).

Camera malfunction was registered on 5 occasions: 3 cameras on LD trips and 2 cameras on ND trips. Occasionally sections of recordings were inadequate e.g. overexposure due to sunlight, camera blocked by an animal, or darkness at nightfall. Average values presented later are corrected for these missing images.

As cameras were located in an upper corner of the study compartments, the limited amount of headroom resulted in side-view more than overhead view. Therefore, viewing of animals in the corners farthest from the camera was frequently obscured by animals in front of the camera, especially when in a standing position. Two examples of camera view are shown in Figure 18.

Figure 18: Examples of camera view with many (left image) and few (right image) visible animals

Examination of pig behaviour was limited to visible animals, which were counted during observation periods. Average number of visible pigs during each 5-minute video scan sample was 7.9 for ND trips, and 7.4 for LD trips. Because the pattern (Figure 19) and number of visible animals apparently did not differ much between LD and ND trips, this was not taken into account during further analysis. However, despite the fact that there was little difference in the number of visible animals, it is recalled here that study compartments held 12 or 13 pigs on ND trips, and 10 pigs on LD trips, and therefore visibility is expressed as percentage of pigs in the compartment.

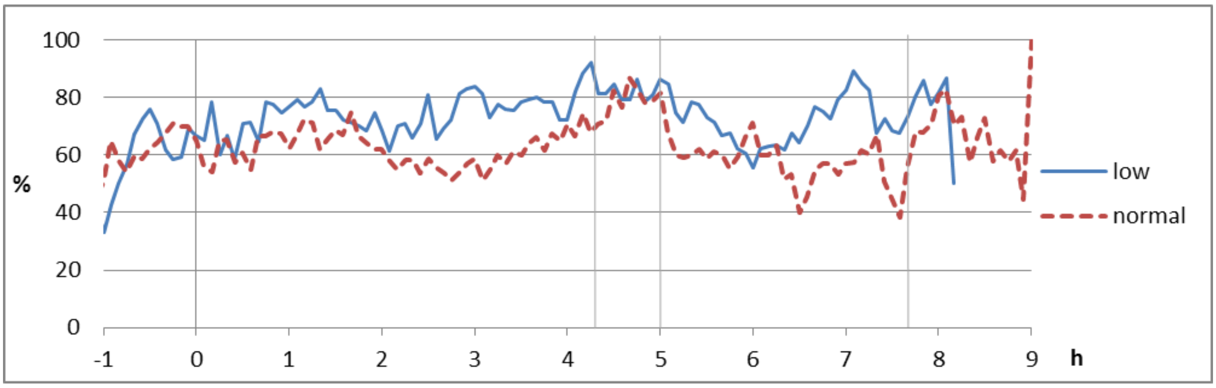

Figure 19: Average pattern of percentage of visible animals on camera during low and normal density trips (time of departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

Irrespective of loading density, it is noted that the number of pigs lying down could be overestimated and the number of pigs standing could be underestimated because visibility is improved when most of the pigs are lying down. This could possibly explain the only notable difference in visibility patterns which occurs between 4:15-5:00h after departure (break). During this period more animals in ND trips were observed to be lying down (see Figure 22).

3.3.1 Activity

Activity was measured at 10 minute intervals as the number of active or moving animals observed during periods of 2 minutes. If an animal became active more than once after a break in activity, this was counted as a new occurrence. Figure 20 shows the average pattern of the number of active animals or bouts of activity (movements) per compartment during 2 minute time frames.

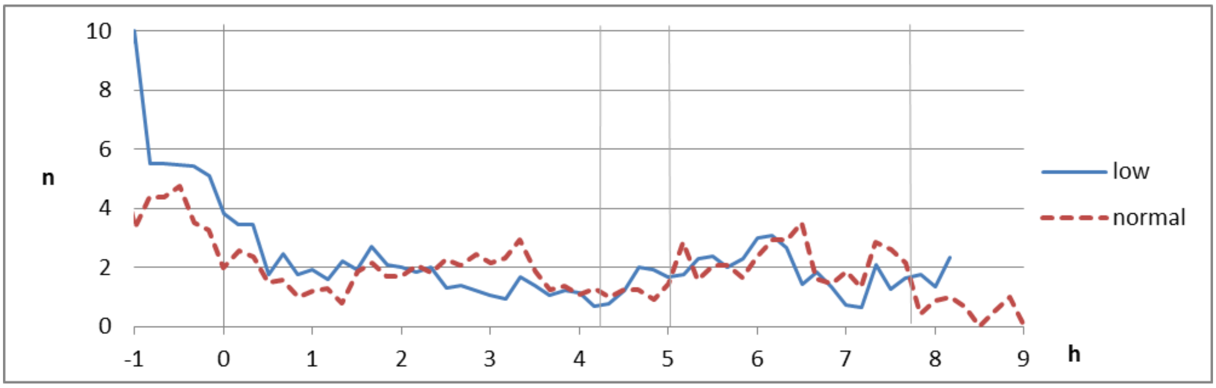

Figure 20: Average pattern of activity (movements /compartment /2 min periods) during LD and ND trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

Greatest activity or restlessness was measured during or immediately following the loading process. Thereafter, pigs appear to become less active or quiet relatively quickly. Before and up to 2h after departure pigs in LD trips appeared to be more active than pigs in ND trips, may be accentuated by the fact that fewer pigs were transported in LD than ND compartments. It is considered likely that LD pigs experienced more freedom of movement than ND pigs. In the period 2-4h after departure there was a tendency for LD pigs to be less active than ND pigs. Thereafter, activity patterns were similar.

Table 4: Trip averages for number of active pigs (movements /compartment /2 min periods)

Density	Trip	Activity
low	2	3.0
	4	1.7
	6	1.8
	7	2.6
Avg. low		**2.2**
normal	1	1.9
	3	2.0
	5	2.5
	8	2.3
Avg. normal		**2.1**

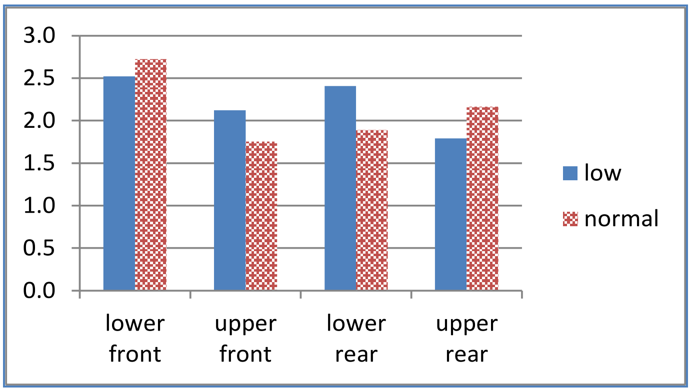

Figure 21: Average number of active pigs (movements /compartment /2 min periods) for each compartment and each density

Differences in activity appear to be larger between individual trips than between the 2 loading densities, as shown in Table 4.
Slight differences in activity were observed between compartments. Animals in the lower front compartment were more active than those in other compartments (Figure 21).

It was very rare that all the pigs in each study compartment were inactive for the whole duration of a 2 min period. During 7 trips this occurred on 1 to 6 occasions (avg. 3.0 times/trip). LD trip number 6 was exceptional because on 10 occasions all pigs in all four study compartments were inactive, this included a 70 minute period of inactivity during (4 samples) and after the halfway break (3 samples). REML analysis revealed that the most activity was registered during leg 1, and in the lower rather than the upper compartments and several interactions could be identified.

3.3.2 Posture

Lying, standing and sitting were observed in each study compartment at 5 min intervals. Average results (as percentage of visible animals) for LD and ND trips are presented in Figure 22 to Figure 24.

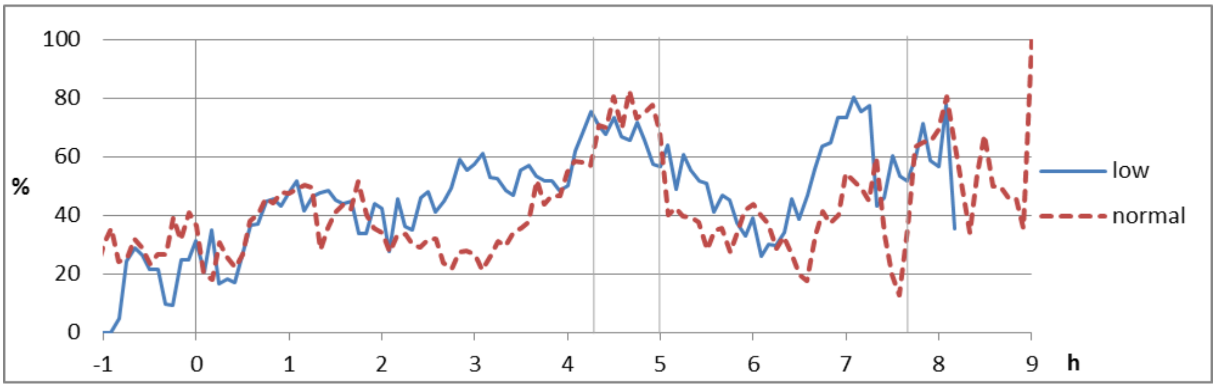

Figure 22: Average pattern of lying pigs lying during LD and ND trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

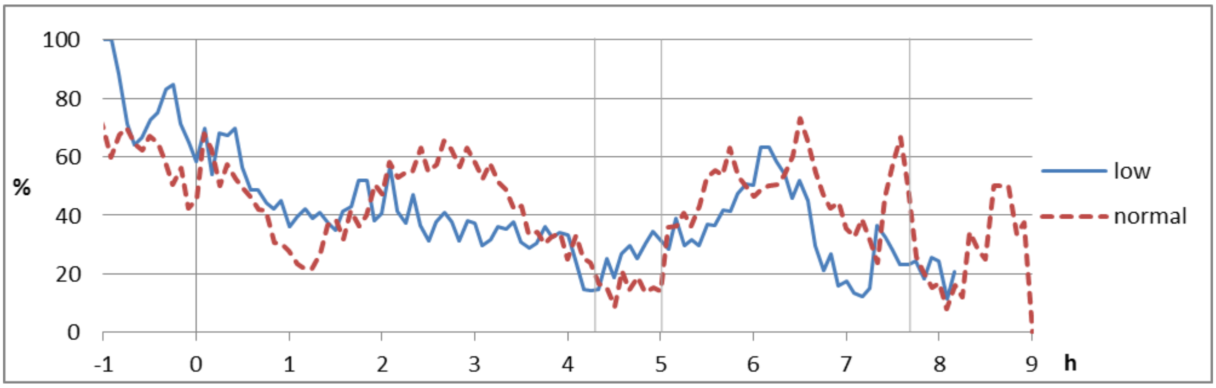

Figure 23: Average pattern of standing pigs standing during LD and ND trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

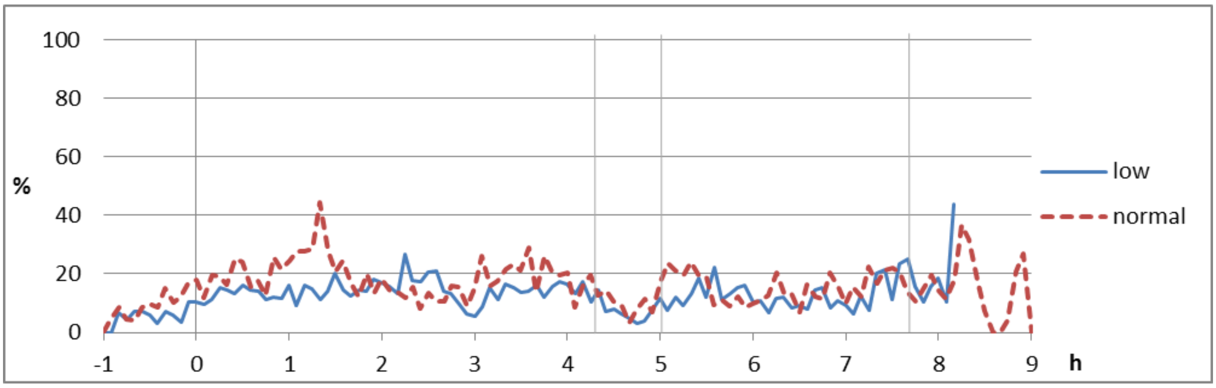

Figure 24: Average pattern of sitting pigs during LD and ND trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

During and immediately after the loading process most of the animals that were visible appear to be standing and their number slowly decreases as the journey progresses. During the second half of both driving periods (legs 2 and 4) more animals in LD trips seem to lie down than in ND trips. Notably around the break and immediately after arrival peaks can be observed in numbers of pigs lying down in ND trips. Seated animals are a minority throughout the trip. Slightly more animals adopt a sitting position at ND than at LD during the first few hours of the journey.

Table 5: Trip averages for numbers/compartment of visible, lying, standing and sitting pigs

Density	Trip	n Visible	n Lying	n Standing	n Sitting
low	2	5.6	1.0	3.9	0.7
	4	8.2	6.2	1.5	0.6
	6	7.8	5.1	1.7	1.0
	7	7.2	3.2	2.9	1.1
Avg. low		**7.4**	**4.3**	**2.3**	**0.9**
normal	1	9.2	5.6	2.5	1.1
	3	8.1	4.8	2.2	1.1
	5	6.2	1.6	3.1	1.6
	8	7.1	3.0	3.0	1.1
Avg. normal		**7.9**	**4.0**	**2.7**	**1.2**

More than half of the visible animals were observed to be in a lying position, whereas approximately one third were standing (Table 5). These data also appear to confirm the earlier impression that as more animals lie down, the overview improves. Numbers of animals lying down on trips 2 (LD) and 5 (ND) were lower than on other trips. It is noteworthy, that there was a large water leakage during trip 2 and that on trip 5 there was traffic congestion (1 hour delay) around Berlin due to heavy rainfall. Numbers lying or standing varies greater between trips than between densities. In particular, both trips 2 and 4 were performed at the lower loading density; nevertheless lying events in trip 2 were highest and lowest in trip 4 for all trips.
As an example, the lying pattern in trip 5 was investigated further in an attempt to explain the low lying counts. It was investigated whether or not slow driving speeds caused by the traffic jam conditions during leg 4 or the relatively long duration of journey were responsible for reductions in lying events. Lying pattern could be related to truck speed (Figure 25), which disclosed that pigs appear to lay down more when the vehicle stops or moves slowly. Therefore, it is considered that traffic congestion was not responsible for the low amounts of lying events during trip 5.

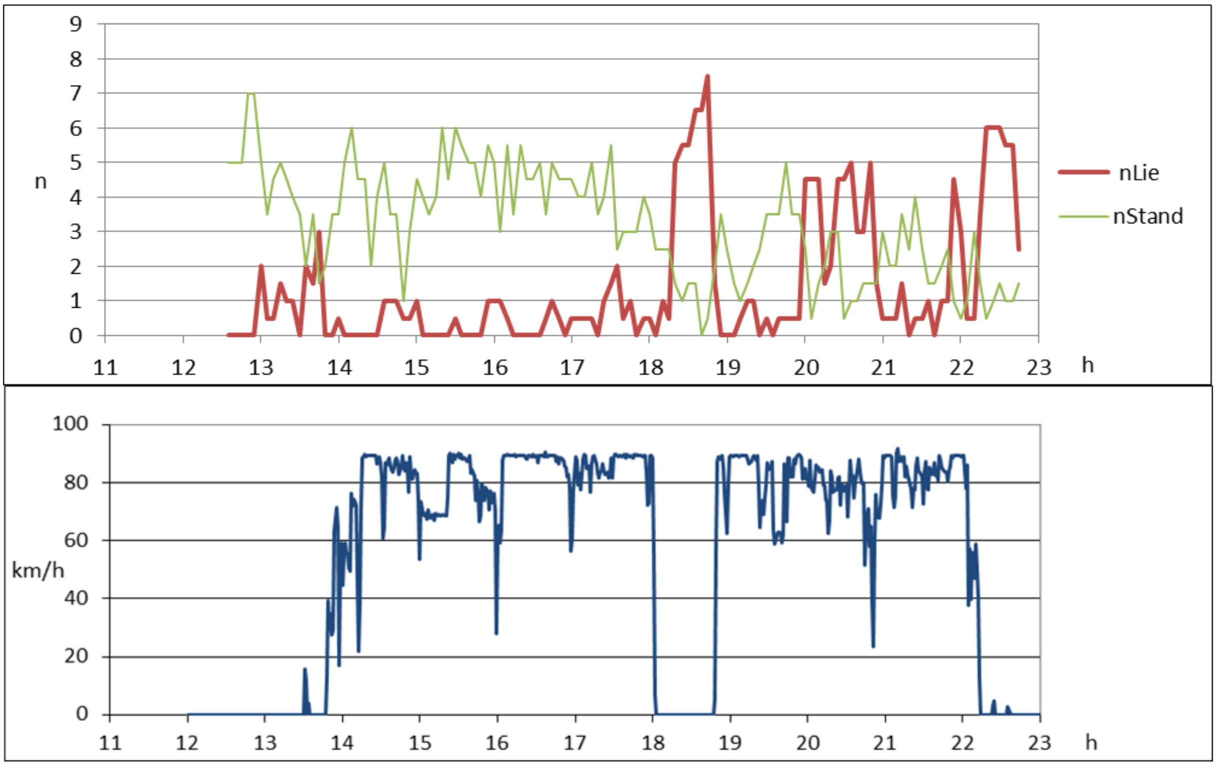

Figure 25: Pattern of lying and standing (number/compartment, upper graph) compared to pattern of driving speed (lower graph) in trip 5

The low levels for lying events clearly originate from leg 2, during which hardly any pigs lay down. This is quite unlike the average pattern illustrated in Figure 22. It remains unclear as to what caused the low amount of lying especially in leg 2 of trip 5. It is considered unlikely that the heavy rainfall provides an explanation, because trip 2 shows an equally low amount of lying while the weather conditions were bright and dry.

Average numbers of lying, standing and sitting animals in each compartment are presented in figures Figure 26 to Figure 28

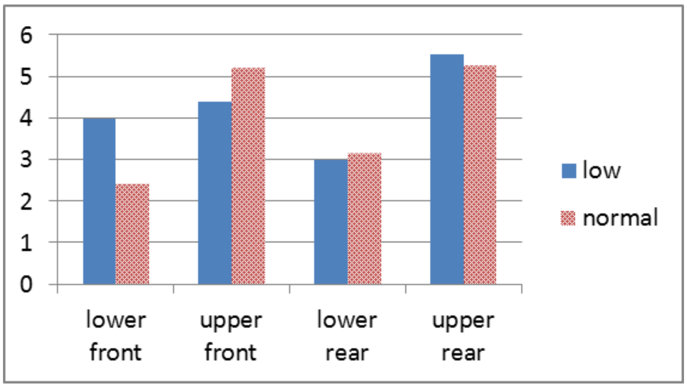

Figure 26: Average numbers of pigs lying in each compartment on LD (low) and ND (normal) trips

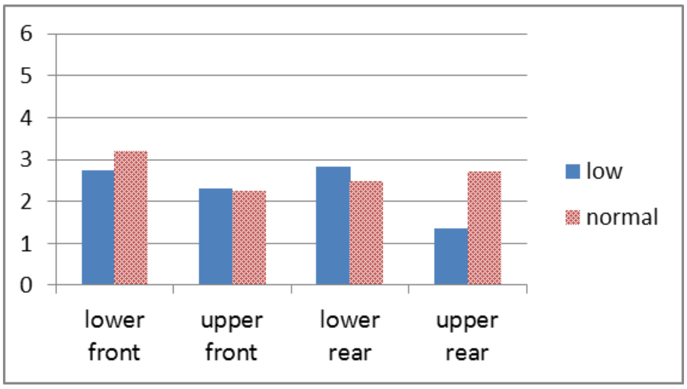

Figure 27: Average numbers of pigs standing in each compartment on LD (low) and ND (normal) trips

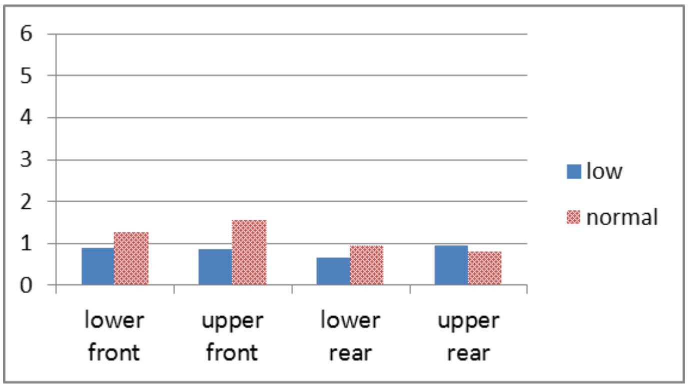

Figure 28: Average numbers of pigs sitting in each compartment on LD (low) and ND (normal) trips

For LD and ND trips more animals were observed lying in the upper compartments than in the lower compartments. The lower front compartment shows a remarkable effect of loading density on the number of pigs lying (most on LD trips), and similar numbers of standing pigs in the upper rear compartment (most on ND trips). The most pigs sitting were observed in the front compartments during ND trips; however numbers and differences were small.
GLMM analyses revealed that in leg 1 significantly fewer lying events occurred than during other legs. There was a gradual decline in standing events as each leg progressed. The animals sat least during leg 1. Several interactions were identified.

3.3.3 Fighting

Incidence and duration of fighting was observed by continuous analysis of full video files of the 4 study compartments.
Excluding 5 instances of camera malfunction, a total of 303 fights with a total duration of 01:57:41h were observed during the 8 trips. Considering an average trip duration of 09:11h (Table 1) this would imply that 3% of journey time was used for fighting somewhere in one of the study compartments. Average duration of a fight was 23 s, however a large variation was observed (range 1 - 166 s). Starting moments of fights were grouped per 30 min (-15 to +15 min) in order to visualize trends in number and duration of fights during the journeys (Figure 29 and Figure 30).

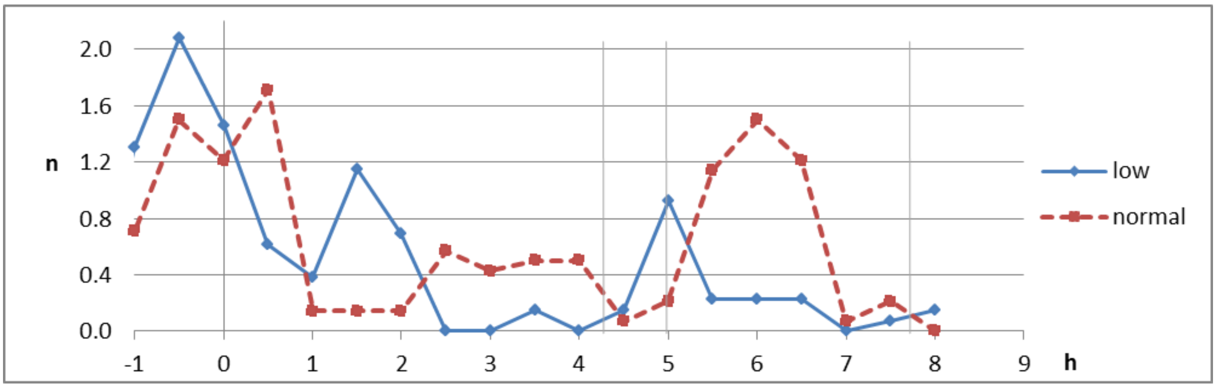

Figure 29: Average pattern of number of fights per 30 min per study compartment during LD (low) and ND (normal) trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

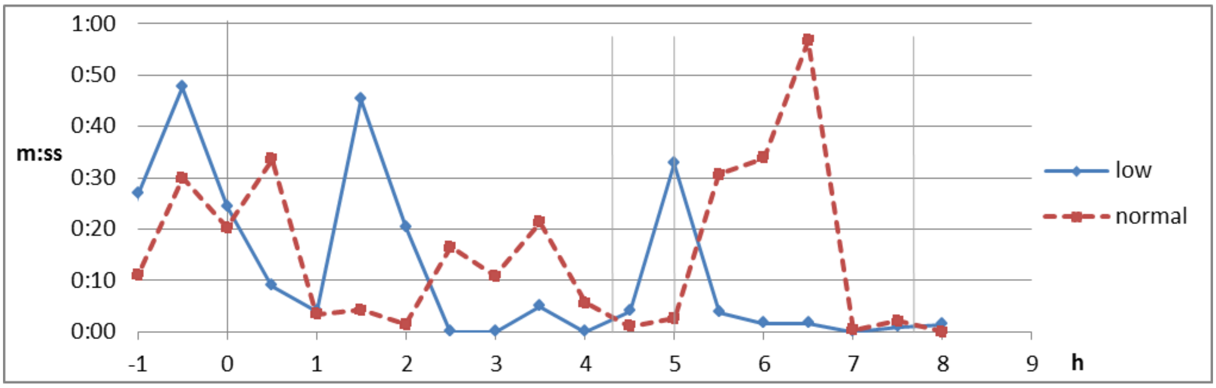

Figure 30: Average pattern of sum of duration of fights per 30 min per study compartment during LD (low) and ND (normal) trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

The sum of fight duration is closely related to the fight counts, even though there was large variation in fight duration. Most fights were observed before and immediately after departure than later in leg 2. Hardly any fights were observed during leg 3 (break), after the break, upon departure in leg 4, a remarkable increase in fighting occurred. Throughout leg 4 more fighting occurred with longer bouts seen in ND trips than in LD trips. At the end of leg 4 and after arrival there was less fighting. Average figures per trip show huge differences in levels of fighting (Table 6), e.g. extreme low levels on trips 2 and 5 and extreme high levels on trips 3 and 8.

Table 6: Total number and total duration of fights per trip

Density	Trip	Total number of fights	Sum of duration of fights (h:m:s)
low	2	8	0:03:10
	4	66	0:27:01
	6	16	0:08:08
	7	46	0:14:37
Avg. low		**34**	**0:13:14**
normal	1	28	0:09:11
	3	70	0:31:27
	5	4	0:01:00
	8	75	0:27:14
Avg. normal		**44**	**0:17:13**

On 3 occasions no fights were observed in the compartment during the whole trip (twice on LD trips, once on ND trip). Additionally, on 3 occasions only a single fight was observed in the compartment during the whole trip (once on LD trip, twice on ND trips). Most fighting occurred in the lower front compartment on ND trip 8: 45 fights with a total duration of 00:19:12 h.

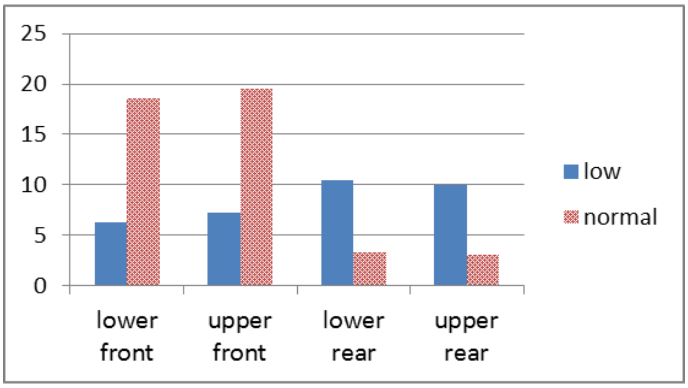

Figure 31: Average number of fights per compartment per trip, during LD (low) and ND (normal) trips

On ND trips by far the most of the fighting appears in the front compartments, whereas fighting on LD trips occurred mainly in the rear compartments (Figure 31). Differences between lower and upper compartments appeared to be relatively small.
REML analysis revealed that incidence and duration of fighting was influenced by leg (1 and 2). Space allowance also displayed a significant influence on fighting. Most of the fighting occurred during the low density trips.

3.4 Heart activity

3.4.1 Measurements

Heart activity was measured using pad electrodes connected to data loggers (8 pigs per trip) and using Polar equipment (4 pigs per trip). Equipment was evenly distributed over the study compartments.
Not all recordings of heart activity were technically successful from start (time of loading) to finish (1h after unloading). In spite of protective measures, equipment appeared vulnerable to destruction or displacement by neighboring pigs. Use of the telemetric loggers resulted in 39 completely successful measurements, 9 incomplete but useable measurements and 16 unusable failures (

Table 7). Measurement with Polar equipment provided 14 completely successful and 18 unusable failures. Lack of or damage to equipment, displacements of equipment, or poor contact between electrodes and skin were often responsible for failures. Sometimes small sections of recordings with the pad electrodes had to be discarded because of disturbances to the signal. All successful and partly successful recordings were used for further analysis.

Table 7: Number of animals with technically successful registrations of heart activity per trip

		Data logger		Polar	
Density	Trip	full	incomplete	full	incomplete
low	2	4	3	2	
	4	2	1	2	
	6	6		1	
	7	6	2	3	
Total low		**18**	**6**	**8**	
normal	1	4	2		
	3	4	1	1	
	5	7		2	
	8	6		3	
Total normal		**21**	**3**	**6**	
grand Total		**39**	**9**	**14**	

Figure 32 presents the averages of heart rate as recorded using pad electrode equipment. The pattern shows a significant development in heart rate as each trip progresses. Initially HR is high and declines in legs 2 and 3, to increase gradually in legs 4 and 5, yet remaining below the initial levels. An increase in heart rate was observed during the halfway break, notably in ND pigs. Average heart rates in ND pigs appear higher than those of LD pigs almost throughout the trips.

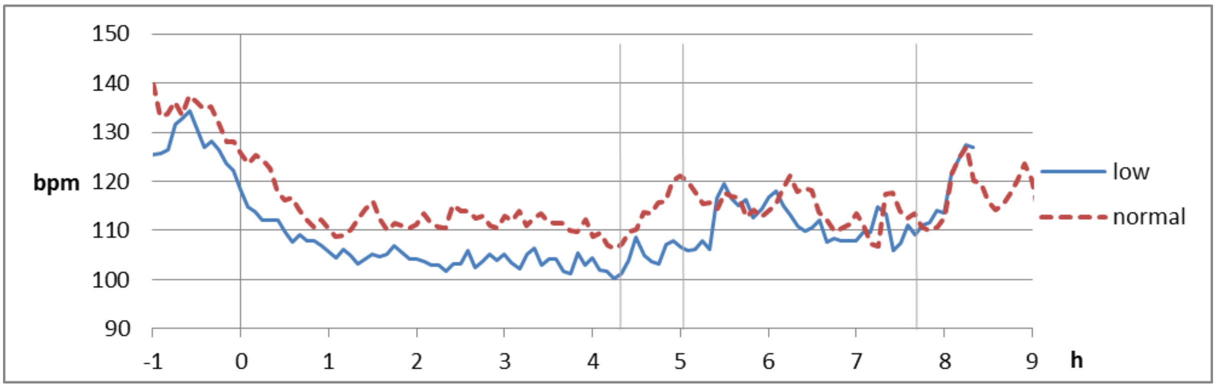

Figure 32: Average pattern of heart rate measured by pad electrode equipment during LD (low) and ND (normal) trips (departure at 0:00h, avg. break after 4:15h, avg. arrival after 7:42h)

The average patterns of heart rate as recorded using Polar equipment are presented in Figure 33. Polar measurements of the heart rate displayed similar general tendency during the trip to those observed with telemetry. However, overall differences between LD and ND pigs are not as apparent as with telemetric loggers.

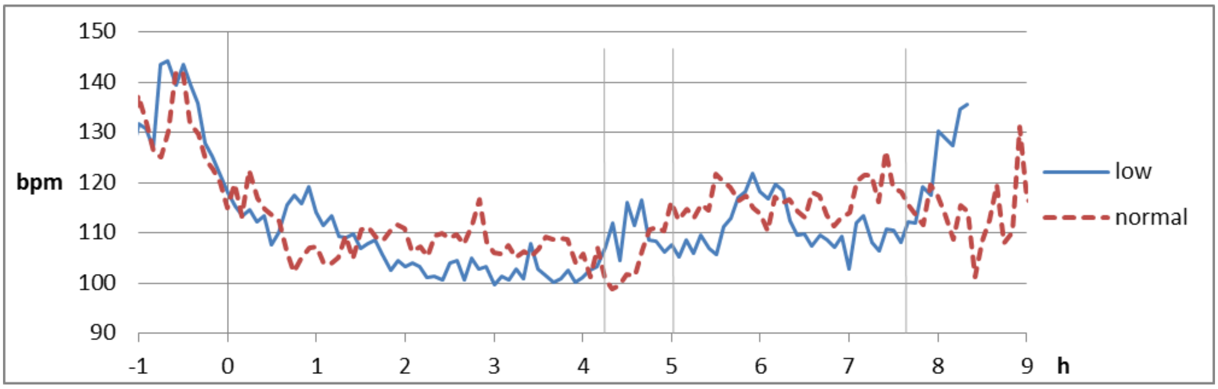

Figure 33: Average pattern of heart rate measured by Polar equipment during LD (low) and ND (normal) density trips (departure at 0:00h, avg. break at 4:15h, avg. arrival at 7:42h)

Average heart rates per leg and per trip (Table 8) confirm the patterns displayed in Figure 32 and Figure 33: highest heart rates during loading, and lowest heart rates during legs 2 and 3, irrespective of loading density. During all legs until arrival pigs consistently show lower heart rate in LD trips than in ND trips.

Table 8: Average heart rates per leg and per trip

		Leg					
Density	Trip	1	2	3	4	5	All legs
low	2	127	110	106	109	124	113
	4	131	104	116	112	118	111
	6	133	108	105	116	117	113
	7	127	103	103	110	117	109
Avg. low		**129**	**106**	**106**	**111**	**119**	**111**
normal	1	140	122	114	110	111	121
	3	133	105	114	109	120	114
	5	123	109	111	113	116	113
	8	139	113	110	122	124	120
Avg. normal		**133**	**112**	**112**	**115**	**118**	**117**

Loading time also had an effect on levels of heart rate. Pigs on the upper deck were loaded first and had more time to acclimatise to conditions on the truck while those on the lower deck (particularly in the rear compartment) had less time to acclimatise before departure. Peaks in heart rate during loading could often be seen in individuals and were more pronounced than those from individuals during unloading.

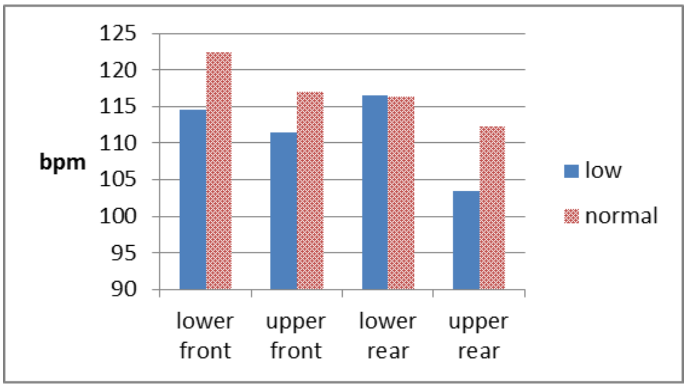

Figure 34: Average heart rates per compartment during LD and ND trips

Differences in heart rates between compartments resembled the level of differences in heart rates between journey legs. On LD as well as ND trips average heart rates of pigs placed in the upper rear compartment were lower than in other compartments (Figure 34). The highest heart rates on LD trips were found in the lower rear compartment, whereas on ND trips these were found in the lower front compartment. Pigs transported on the lower deck generally tended to have higher heart rate values, as well as pigs placed in the front of the vehicle.

3.4.2 Heart rate variability

As opposed to the Polar equipment, the data loggers registered each individual heartbeat, which made it possible to calculate heart rate variability (HRV) parameters for pigs that had been equipped with data loggers. HRV parameters are calculated over a series of inter-beat intervals, i.e. over a chosen period of time. An HRV parameter value for the total period encompassing all legs does not necessarily need to be equal to the average of parameter values of the individual legs.
HRV parameters were calculated per pig for the total period of each leg and for the total period encompassing all legs together from loading until unloading
The parameters that were calculated are:

- SDRR standard deviation of inter-beat intervals
- RMSSD root mean square of successive differences in inter-beat intervals
- LF/HF ratio of the power in the low frequency band to that in the high frequency band
- HF/Total ratio of the power in the high frequency band to the total of powers in all bands

Figure 35 and Figure 36 present the averages for HRV parameters per leg (left) and per compartment (right) during LD and ND trips. Figure 35 also shows the sum total HRV levels calculated for all legs (‘all’); however these data are not included in the graphs to the right or in Table 9. This to avoid overlap of periods on which averages are based.

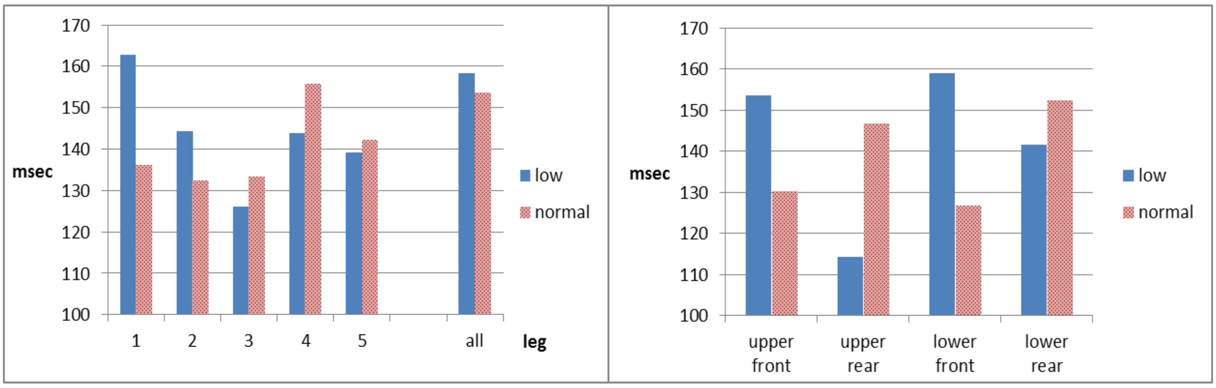

Figure 35: SDRR of inter-beat intervals per leg (left) and per compartment (right) during LD and ND trips

SDRR values of all inter-beat intervals in a chosen period (as a measure of variation in heart rate) (Figure 35) are higher during legs 1 and 2 and lower during legs 3 to 5 on LD than on ND trips. Lowest values are found during leg 3 (halfway break), and relatively high values during leg 4. SDRR levels were highest in leg 1 for pigs on LD trips. During the whole journey SDRR values were highest on LD trips. Averages per compartment differ between LD and ND trips: highest SDRR on LD trips were found in the front compartments whereas highest SDRR on ND trips were observed in the rear compartments. Journey leg and placement (compartment) in the vehicle appear to produce larger effects than loading density.

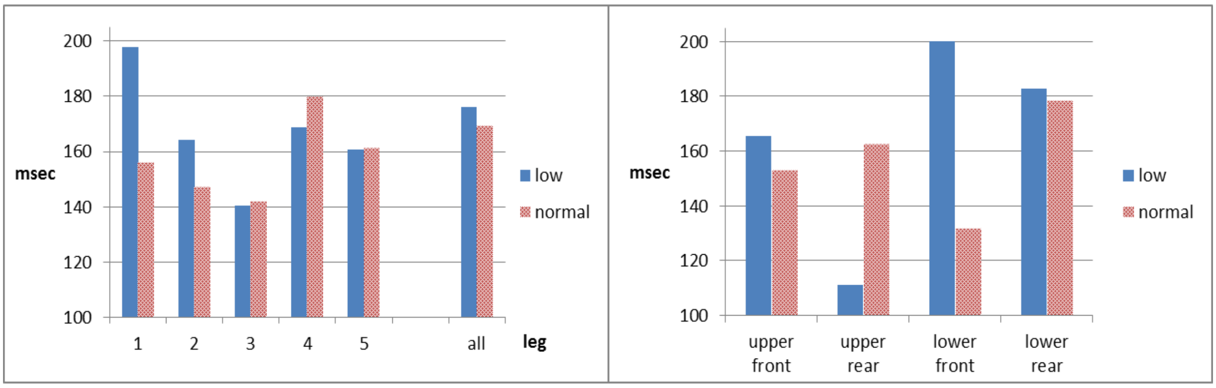

Figure 36: RMSSD of inter-beat intervals per leg (left) and per compartment (right) during LD and ND trips

RMSSD values of successive inter-beat intervals in a chosen period (as a measure of variation in successive heart beats, Figure 36) show comparable patterns to those presented for SDRR, for legs and compartments. Differences in RMSSD between densities, legs and compartments are similar to those found for SDRR. However, the highest RMSSD on LD trips were observed in the lower compartments. Journey legs and placement (compartment) appear to have a larger effect than loading density.

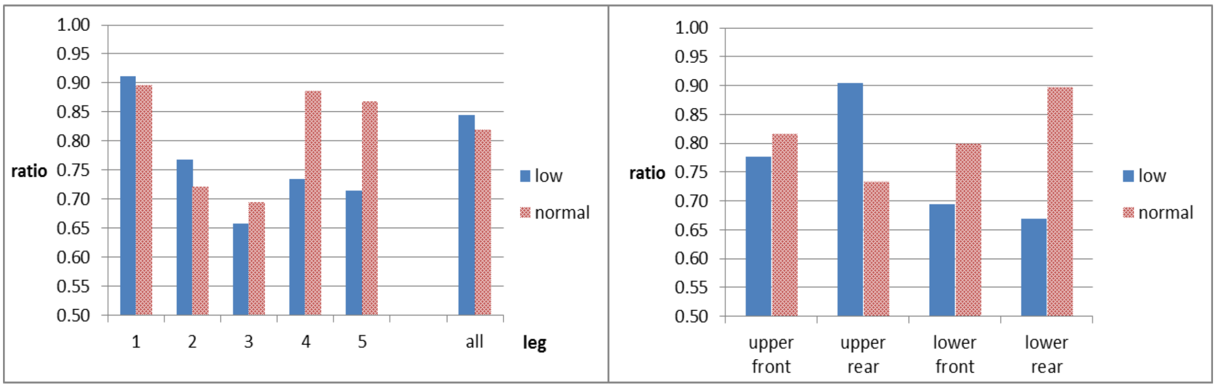

Figure 37: LF/HF power ratios per leg (left) and per compartment (right) during LD and ND trips

LF/HF frequency band power ratios of inter-beat intervals in a chosen period are considered an important indicator of sympathetic activity (Figure 37). These display large variation between legs. During the first part of journey, LD pigs show higher values than ND pigs, and this appears to be reversed during the second half of the journey. Again lowest values were found during leg 3. During the whole journey pigs travelling at LD have slightly higher LF/HF ratios than those transported at ND. It would appear (Figure 37; right) that LF/HF ratios are highest in the upper compartments on LD trips, whereas during ND trips the highest values were observed in the lower rear compartment. Once again journey leg and placement (compartment) appear to have greater effect than loading density.

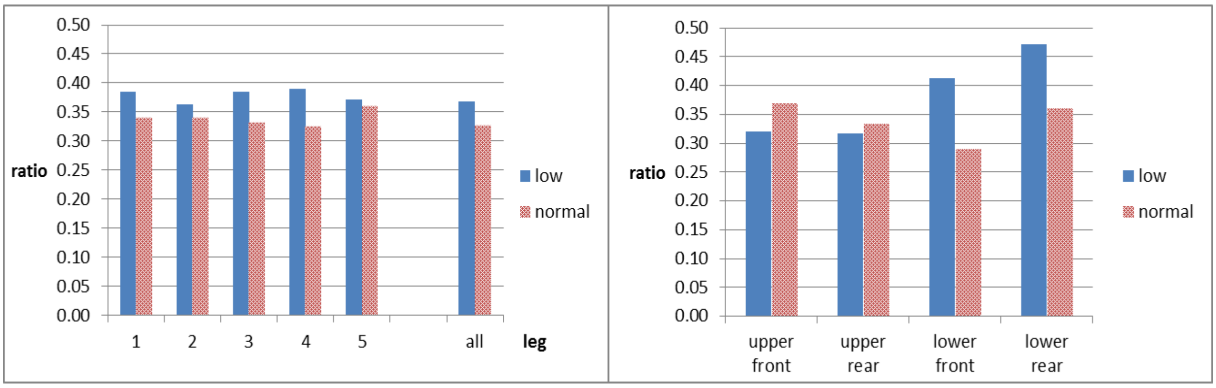

Figure 38: HF/Total power ratios per leg (left) and per compartment (right) during LD and ND trips

HF/Total frequency band power ratios of inter-beat intervals provide an indication of vagal activity. Results show (Figure 38; left) similar levels and with little variation between space allowances for individual legs or the whole journey. Pigs transported at LD consistently display higher HF/Total power ratios than ND pigs. It appears (Figure 38; right) that HF/Total power ratios on LD trips were lowest in the upper compartments, whereas on ND trips the lowest values were recorded in the lower front compartment. Although relatively small, the effect of space allowance appears to be larger than effects of legs or equivalent to that from placement (compartment) in the vehicle.

Table 9: Averages of HRV parameters during each trip

Density	Trip	SDRR (msec)	RMSSD (msec)	LF/HF ratio	HF/Total ratio
low	2	126	123	0.77	0.30
	4	180	210	0.89	0.40
	6	122	149	0.74	0.41
	7	171	214	0.68	0.41
Avg. low		**143**	**166**	**0.76**	**0.38**
normal	1	119	128	0.83	0.31
	3	193	227	0.93	0.34
	5	172	208	0.61	0.43
	8	83	75	0.94	0.26
Avg. normal		**140**	**157**	**0.81**	**0.34**

Differences in HRV parameters between trips (Table 9) were larger than the differences between loading densities. Trip 3 in particular, displays high values for most of the HRV parameters. Whereas, trip 8 displays the lowest values in time domain measures of variability (SDRR and RMSSD), the highest LF/HF ratio and the lowest HF/Total power ratio of all trips.

3.5 Body temperature

Body temperature was recorded per minute by thermo-sensors inserted into the vagina of 12 monitor pigs per trip. Results show that 60 of the 96 registration attempts were technically successful and fully usable. The majority of the failures were due to sensors being ejected from the body cavity during the journey.
Average body temperature recorded for pigs transported at LD was lower than those at ND for almost the whole journey (Figure 39). Figure 39 contains extra data at both ends of the graph to include body temperatures prior to loading and after unloading.

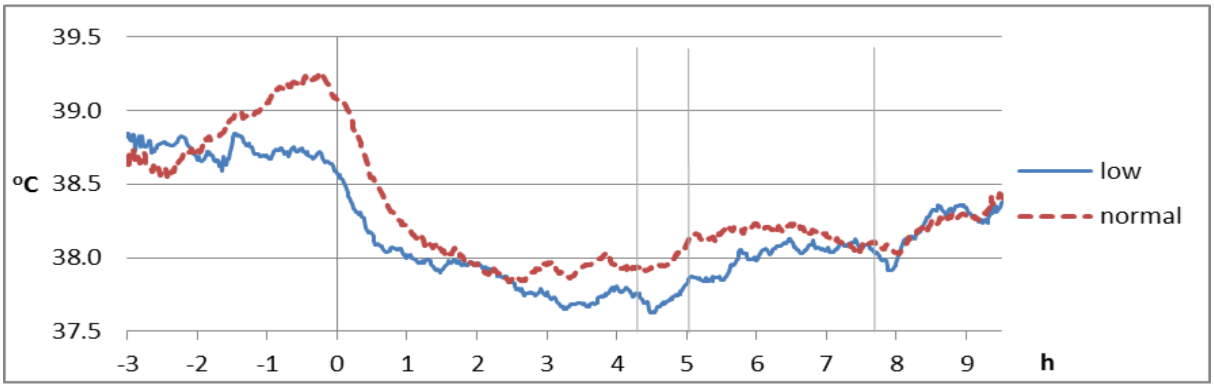

Figure 39: Pattern of average body temperature during LD and ND trips

Loading was generally performed in the period from -1:30h to -0:30h implying that loading density can take effect from -1:30h onwards. Around 1:30h before departure body temperatures on ND trips tended to rise abruptly from values prior to this. Whereas, on LD trips this did not appear to be the case. Within 2 to 3h after departure body temperatures on both LD and ND trips fell by approximately 1.0 °C (maximum), with a greater decrease in ND pigs than in LD pigs. From 3h after departure onwards body temperature remained relatively stable. During the break and after arrival (stationary periods) body temperatures tended to increase slowly. A distinctive rise in body temperature was also observed in leg 4 of the journey. At the same time, outer and inner truck temperatures tended to fall as evening approached. Prior to loading (t < -1:30h) and after unloading (t > 8:30h) density effects appear to be absent. During these periods study pigs were not held under different study conditions but were kept as a single group indoors i.e. farm pen or slaughterhouse lairage.
Average pre-transport body temperature (-3h to -1h) was 38.7°C in LD pigs and 38.8°C in ND pigs. Post-transport body temperature (8:15h to 9:30h) averaged 38.3°C. Lowest average body temperature was reached 4:30h after departure in LD pigs (37.6 ° C) and 2:30h after departure in ND pigs (37.8 ° C).

Table 10: Average, minimum and maximum body temperature (°C) in pigs during trips

Density	Trip	Average	Minimum	Maximum
low	2	38.15	35.63	39.38
	4	38.19	36.75	39.25
	6	37.95	34.13	39.88
	7	37.98	36.13	39.50
Avg. low		**38.04**	**34.13**	**39.88**
normal	1	38.27	36.13	41.13
	3	38.30	35.88	42.13
	5	38.17	36.08	39.88
	8	38.35	36.88	40.63
Avg. normal		**38.27**	**35.88**	**42.13**

Average body temperatures per trip (Table 10) appear to indicate an overall difference between LD and ND trips: body temperatures were almost always lower on LD trips. Trips 1 and 2 that were performed on warm summer days did not display the highest average body temperatures. Minimum and maximum values displayed in Table 10 indicate the range encountered in individual measurements during each trip.

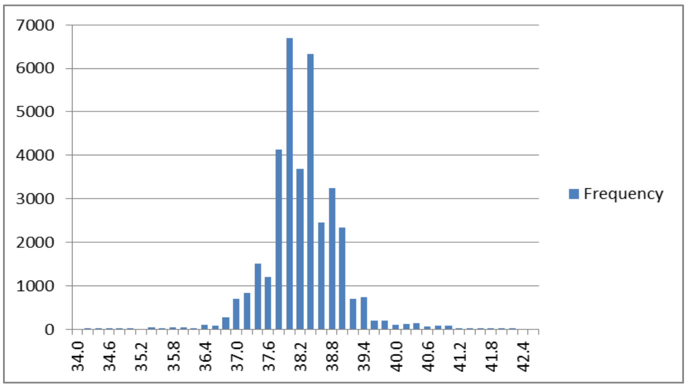

Figure 40: Histogram of body temperatures measured in all trips

Care was taken not to include measurements that could be other than inner body temperatures e.g. near either end of the measuring period, or from sensors that could have been ejected from the body. The majority of body temperatures (95.36% of all samples) fell in the range from 37.0 - 39.5 ^{o}C (Figure 40). However, incidental deviations from this range were large, from as low as 34.1 ^{o}C to as high as 42.1 ^{o}C. Temperatures below 37.0 ^{o}C were recorded on 1018 (2.30%) occasions: 645 of these were on LD trips involving 10 pigs and 373 on ND trips involving 7 pigs. Most of these were recorded 3-5h after departure. Temperatures above 39.5 ^{o}C were recorded on 1035 (2.34%) occasions: 27 of which were on LD trips involving 2 pigs and 1008 on ND trips involving 15 pigs. Most of these were recorded -1.5h to +0.5h around departure time. This shows that extreme low body temperatures occurred more often on LD trips, whereas extreme high body temperatures occurred almost solely on ND trips.

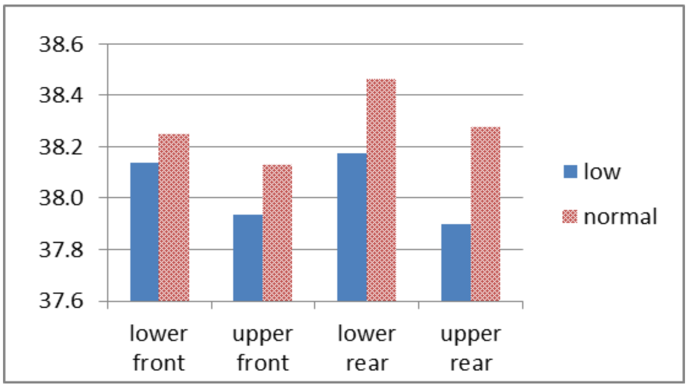

Figure 41: Average body temperature per compartment during LD and ND trips

Average body temperatures per compartment (Figure 41) appear to illustrate an overall difference between LD and ND trips: i.e. lower body temperatures in LD than in ND pigs in all compartments. The lowest body temperatures were recorded in the compartments on the upper deck per stocking density. While the overall level of body temperature was generally higher for ND than LD trips. The highest and lowest body temperatures were recorded in pigs transported in the lower and upper rear compartments respectively.

3.6 Blood parameters

Blood samples were taken prior to loading and after unloading from 12 pigs on each trip, i.e. 3 pigs in each of the 4 study compartments. Levels of blood parameters were analyzed, and response values (differences in levels after transport minus levels before transport) were calculated.

3.6.1 Blood analysis

Average values for blood parameters are presented in Table 11, according to time of sampling and loading density,

Table 11: Blood parameter averages at time of sampling per treatment group (low or normal loading density)

			sample time and loading density					
			Before transport		After transport		After-Before	
parameter	**abbr.**	**unit**	**low**	**normal**	**low**	**normal**	**low**	**normal**
albumin	Alb	g/L	27.8	27.7	29.6	29.0	1.7	1.3
protein	Pro	g/L	75.0	75.3	77.3	77.4	2.3	2.1
globulin:albumin	Glo:Alb		1.72	1.74	1.65	1.71	-0.07	-0.03
glucose	Glu	mmol/L	4.99	4.89	5.30	5.26	0.31	0.37
L-lactate	Lac	mmol/L	2.90	3.01	2.08	2.41	-0.82	-0.60
lactate dehydrogenase	LDH	U/L	820	1139	854	1564	34	425
aspartate aminotransferase	AST	U/L	25.1	35.6	29.8	48.5	4.6	12.8
creatine kinase	CK	U/L	1142	1657	1912	3939	770	2282
cortisol	Cort	ng/mL	41.3	37.0	34.6	49.0	-6.2	12.1
haematocrit	Ht	%	36.4	35.2	31.8	32.8	-4.6	-2.3

3.6.2 White blood cells

Averages for white blood cell counts are given in Table 12 for each treatment group (low or normal space allowance) from blood samples taken prior to or after completion of the trips.

Table 12: Average white blood cell counts at time of sampling per treatment group (low or normal loading density)

		sample time and loading density					
		Before transport		After transport		After-Before	
parameter	**unit**	**low**	**normal**	**low**	**normal**	**low**	**normal**
lymphocytes	%	68.1	66.3	43.5	44.6	-24.6	-21.7
neutrophils_segmented	%	21.2	23.2	45.0	43.1	23.9	19.9
neutrophils_banded	%	5.1	5.0	9.1	10.1	3.9	5.1
basophils	%	0.5	0.7	0.2	0.3	-0.3	-0.4
eosinophils	%	3.7	3.7	0.9	0.8	-2.8	-2.9
monocytes	%	1.4	1.1	1.3	1.1	-0.1	0.0

3.7 Water usage

Water usage was recorded for the truck as a whole by measuring the outflow from the water tanks in litres per minute. Therefore, it was not possible to distinguish drinking from spillage, and compartment effects could not be studied. Valid recordings were available for five trips. On trips 3 (ND) and 7 (LD) recording failed. Examination of data from trip 2 (LD) disclosed an extremely high flow of water for almost the whole of leg 2 and zero flow thereafter, the likely cause being water leakage followed by empty tanks.
Because of high variations in water usage rate per minute, water use was averaged per periods of 10 min (-5 to +5 min) in an attempt to elucidate trends in water usage during the journeys (Figure 42).

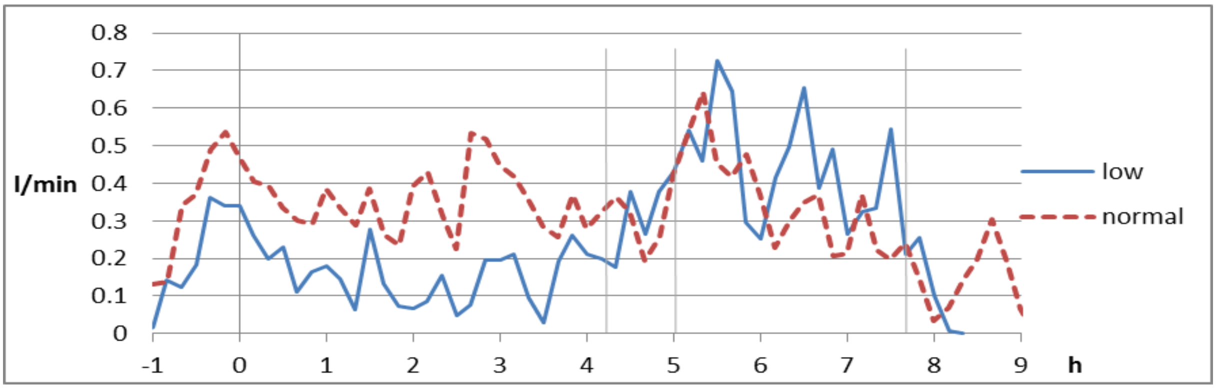

Figure 42: Pattern of water usage during transportation displayed as average per loading density (low or normal)

Pigs did drink during the journeys. Average water use on ND trips appears to have been more evenly distributed during the journey than on LD trips. However during trip 1 the water usage pattern appeared to resemble the overall pattern for low density. Water use on LD trips appears to be relatively low during leg 2 and high during leg 4; trip 4 was most profound in this pattern. No specific changes in water usage were apparent during the break or after arrival at the slaughterhouse. The increase in average water use during ND trips after 8:45h was caused by and coincides with a relatively long waiting time at the unloading platform during trip 5. During this trip there was also a delay for almost an hour in leg 4.
Rate of water flow (l/m) for whole journeys was lower on LD than on ND trips (Table 13).

Table 13: Average water flow rate expressed in liters per minute, per journey and per animal

Density	Trip	l/min	l/trip	l/pig
low	4	0.20	106.4	0.71
	6	0.30	157.5	1.05
Avg. Low		**0.25**	**139.2**	**0.93**
normal	1	0.13	74.0	0.39
	5	0.34	204.1	1.07
	8	0.50	263.8	1.39
Avg. normal		**0.32**	**175.0**	**0.92**

Variation in water usage is high between trips. Water usage on trip 1 was surprisingly low considering the warm weather conditions. Because the journeys differed in duration and numbers of pigs involved, the rate of water usage, expressed as l/min was used to calculate the water usage per trip and per pig. Results then indicate a higher usage during ND trips which could be related to the fact that these trips involved more animals. On the other hand, average water use per pig per trip was approximately 1 liter independent of loading density, although this varied considerably between trips. Average daily water intake of finisher pigs near slaughter is 3-5 l/min.

3.8 Statistical significance

The aim of this study was to investigate the effects of loading density on pigs transported for slaughter, and whether the location in the truck is of relevance. Transportations with low and normal loading densities were carried out. Location in the truck was examined in four study compartments: lower-front, upper-front, lower-rear and upper-rear. In statistical testing however these compartments were crosswise paired into two separate factors: Front vs. Rear, and Lower vs. Upper. Where applicable (i.e. behaviour and cardiac responses), the phase of the journey (leg) was also included in the model, as well as pre-transport levels for parameters in blood (as covariate), including white blood cell counts. Statistical significance was assessed using REML and GLMM as outlined previously in paragraph 2.3. Results for effects of main factors and first level interactions for groups of parameters are presented in Table 14 and Table 15.

Table 14: P-values indicating significance of main factors and first level interactions for behavioural and physiological parameters. Empty spaces indicate non-significance; '≈' indicates trend approaching significance (0.05<P<0.10)

P-values	Main factors				Interactions					
Parameter	Leg	Density	Front-Rear	Lower-Upper	Leg * Density	Leg * Front-Rear	Leg * Lower-Upper	Density * Front-Rear	Density * Lower-Upper	Front-Rear * Lower-Upper
Behaviour										
Activity	< 0.001			< 0.001	0.049	0.037	< 0.001			0.032
%Lying of Visible	< 0.001				≈		≈			
%Standing of Visible	< 0.001						0.004			
%Sitting of Visible	< 0.001				0.038		0.021			
Number of Fights	< 0.001	≈					0.015			
Duration of Fights	< 0.001	0.047					0.027			
Heart rate										
Heartrate (logger)	< 0.001			≈			≈			
Heartrate (Polar)	< 0.001		0.004		≈					
Heart rate variability (legs and total journey)										
SDRR								≈		
RMSSD										
LF/HF ratio	0.007								≈	
HF/Total ratio			≈						0.010	
Heart rate variability (multiple 5min periods)										
SDRR	0.025			0.013						≈
RMSSD				0.002				0.030	0.012	0.002
LF/HF ratio				0.004						
HF/Total ratio				0.014					0.006	0.006
Body temperature										
Body temperature	< 0.001	0.049			≈	0.030				

Table 15: P-values indicating significance of main factors and first level interactions for blood parameters. Empty spaces indicate non-significance; ≈ indicates trend approaching significance (0.05<P<0.10)

P-values	Main factors				Interactions		
Parameter	Density	Front-Rear	Lower-Upper	Covariate (pre-test values)	Density * Front-Rear	Density * Lower-Upper	Front-Rear * Lower-Upper
Blood (post-test values)							
Alb				< 0.001			
Pro				< 0.001			≈
Glo:Alb				< 0.001		≈	
Glu							
Lac							
LDH							
AST	≈						
Ck	≈						
Cort				≈			
Ht				≈			
White Blood Cells							
lymphocytes				< 0.001			
neutrophil_segmented				0.003			
neutrophil_banded				0.006			
basophils					< 0.001		0.011
eosinophils							0.018
monocytes							≈

Journey leg has a major effect on almost all behavioural and cardiac parameters, and effects of legs can be observed in the figures depicting the average patterns presented previously for each parameter. However, legs are not the focus of this study - by definition they were not comparable - and the same holds true for the pre-test values in blood parameters, including white blood cell counts. It is observed that for a number of blood parameters the pre-test values have a strong influence on the post-test values (see significant effects of covariates).

Main effects of loading density were found for the following parameters:

- Number of fights: highest values in LD pigs (approaching significance)
- Duration of fighting: highest values in LD pigs
- Body temperature: lowest values in LD pigs
- AST: lowest values in LD pigs (approaching significance)
- CK: lowest values in LD pigs (approaching significance)

Furthermore, the P-value for cortisol (0.105) was just outside the range approaching significance and overall levels for predicted means differed considerably: 33.6 ng/ml in LD versus 49.9 ng/ml in ND pigs.

Main effects were also found for placement to the front or rear of the vehicle:

- Heart rate (by Polar equipment): highest values in front compartments
- HF/Total power ratio: highest values in rear compartments (approaching significance)

Placement on the upper or lower deck of the vehicle had major effects on:

- Activity: highest values in lower compartments
- Heart rate (by data logger equipment): highest values in lower compartments (approaching significance)
- Heart rate variability (multiple 5 min periods):
    - SDRR: highest values in lower compartments
    - RMSSD: highest values in lower compartments
    - LF/HF ratio: lowest values in lower compartments
    - HF/Total power ratio: highest values in lower compartments

Interactions with the factor density were found for the following parameters:

- Interactions between densities and Legs.
    - Activity: during leg 1 higher activity in LD pigs than in ND pigs. No differences were observed during the other legs.
    - Lying behaviour (approaching significance): different patterns were observed over legs, however no differences were observed between LD and ND pigs during any of the legs.
    - Sitting behaviour: different patterns over legs, however no differences between LD and ND pigs during any of the legs.
    - Heart rate by Polar equipment (approaching significance): different patterns over legs, No differences between LD and ND pigs during any of the legs.
    - Body temperature (approaching significance): during legs 1 and 3 lower body temperatures were observed in LD than in ND pigs. No differences during other legs.
- Interactions between densities and longitudinal placement (front or rear).
    - SDRR (legs and total journey): higher values in rear than in front compartments, for ND pigs only (approaching significance)
    - RMSSD (multiple 5 in periods): higher values in rear than in front compartments, for LD pigs only
    - Basophils: no differences between LD and ND pigs in any of the compartments; however higher values in rear compartments for ND pigs only
- Interactions between densities and vertical placement (lower or upper deck).
    - LF/HF ratio (legs and total journey): higher values in LD than in ND pigs on upper deck; lower values in LD than in ND pigs on lower deck (approaching significance)
    - HF/Total power ratio (legs and total journey): higher values in LD than in ND pigs on lower deck; lower values in LD than in ND pigs on upper deck
    - RMSSD (multiple 5 min periods): higher values in LD than in ND pigs, in lower compartments only
    - HF/Total ratio (multiple 5 min periods): higher values in LD than in ND pigs, in lower compartments only
    - Globulin/Albumin ratio (approaching significance): lower values in LD than in ND pigs on lower deck. No differences in other compartments.

Occasionally interactions were found between journey leg and longitudinal placement in the vehicle (front or rear compartments):

- Activity: during leg 5 there was more activity in rear compartments. No differences were observed during other legs.
- Body temperature: during legs 2 and 3 lower body temperatures were observed in the front compartments. No differences were observed during the other legs.

Many interactions have been indicated between journey leg and vertical placement in the vehicle (lower or upper deck) especially for behavioural parameters. However, these were found to originate mainly from legs 1 and 5 during which the variation in logistics of loading and unloading on upper and lower decks was of utmost importance.

Finally, interactions between longitudinal (front-rear) and vertical (lower or upper deck) placement in the vehicle were found for the parameters:

- Activity: higher values in lower-rear than in upper-front compartment.
- SDRR (multiple 5 min periods): higher values for pigs on lower deck than on upper deck, in rear compartments only (approaching significance).
- RMSSD (multiple 5 min periods): higher values for pigs on lower deck than on upper deck, in rear compartments only.
- HF/Total ratio (multiple 5 min periods): higher values for pigs on lower deck than on upper deck, in rear compartments only.
- Protein: higher values in lower-rear and upper-front than in upper-rear and lower-front compartments (approaching significance).
- Basophils: lower values in lower-front compartment; higher values in upper-rear compartment.
- Eosinophil's: higher values in lower-front and upper-rear compartments; lower values in upper-front compartment.
- Monocytes: lower values in lower-rear compartment than in all other compartments (approaching significance).

Second and third level interactions will not be discussed.

4 Discussion

4.1 Execution

This study comprised eight road journeys performed at one of two stocking densities with slaughter pigs during different seasonal conditions. Our investigation of seasonal variation was limited by the availability of commercial transportations. Therefore, we were only able to monitor transport during the summer and autumn. All journeys were completed successfully although, road and traffic conditions differed between journeys. In spite of protective measures not all heart activity recordings were technically successful from start to finish of a journey. Equipment appeared vulnerable to destruction or displacement by companion animals. Failure or incomplete recordings of heart activity was mainly caused by damage and displacement of electrodes. However, it appears that there sufficient data was collected to provide statistical support for our findings.
Regarding the journeys themselves, for purpose of analysis we identified 5 legs (phases) within each journey (trip). It remains debatable whether or not we have sufficient information concerning the loading and unloading periods which have in the past (Van Putten & Elshof, 1978) been singled out as particularly stressful procedures for animals.

4.2 Behavioural response

During and immediately after loading pigs are most active. After beginning their journey pigs gradually become less active. During the first 2 hours of the journey pigs transported at the lower density were more active than those loaded at the normal space allowance. However, journey conditions appeared to have a larger effect than stocking density.
Although stocking density did not significantly affect the percentage of pigs laying down at certain times during the journeys, it appears that a larger percentage (60%) of pigs transported at low density is laying down 1 hour prior to the lunch break than when transported at the normal density (20%) . From this it can be assumed that during driving pigs need to rest and lay down if they have enough space.
During the lunch break, when the truck is stationary, up to 80% of the animals are laying down irrespective of stocking density. Therefore, it would appear that if the truck is not moving it is easier for more animals to find enough free space to rest whereas during driving this appears to be more difficult, particularly at higher stocking densities.
Fighting occurred in all trips at both stocking densities. Most fights were observed at loading, in the first two hours after departure and after departure following the lunch break. The duration of the fights was significantly longer at normal stocking densities compared to the low density journeys. Furthermore, more fighting incidents are observed in the normal density journeys after the lunch break, whereas incidence of fighting in the LD groups after the beginning of transportation. This is regarded as a successful attempt to establish rang order in groups with more living space. However, there appears to be an effect of location in the truck on fighting behaviour but this is not consistent between stocking densities. During normal density journeys by far the most incidences of fighting occurred in the front compartments whereas during low density journeys most fighting was in the rear compartments. Positioning in the vehicle and the problems in related to (poor) ventilation have been suggested in earlier studies (Meat & Livestock Commission, 1993; Warriss et al 2006) as having a potential role to play in fighting behaviour but due to the contradictory evidence between location and stocking density found in this study, this can't be confirmed. However, it should be realized that all groups were mixed so groups comprised individuals unknown to each other.

4.3 Physiological response:

In the 3 hour period before transportation, body temperature of all pigs was higher than at any time while the vehicle was moving. The increased body temperature baseline is as expected and is considered to be the result of handling and restraining the pigs during instrumentation and measuring of the pigs. More interesting is the increase in body temperature during the actual loading activity in the normal density groups compared to the low density groups. Since all groups of animals are treated equally during the experiments the increase during loading is considered to be related to higher activity or to stress associated with limitation of living space in the transport vehicle. During transportation the body temperature decreased consistently within 1 hour to normal baseline values

but, at the higher loading densities body temperature remained slightly higher than at low loading densities
A similar trend to body temperature was observed for heart rate. Hart rate was considerably increased compared to normal baseline values during the loading period. As soon as the animals are all loaded and the vehicle is closed, heart rate starts to decline consistently to normal baseline values. Although not significant, heart rate remains higher in the normal loading density groups compared to the low density groups. A remarkable observation is the increase in heart rate during the lunch break of the driver. Shortly after parking the vehicle heart rate starts to increase however, the increase is more vigorous for pigs at higher loading densities. After a short period body temperature of all pigs starts to follow the heart rate, which also increases during the break. This increase in heart rate and body temperature is not suspected when observing the resting behaviour of the pigs. Since more than 80% of the pigs are laying down during the driving brake it would be expected that body temperature and heart rate remained at normal levels. When observing the environmental conditions during driving and resting it is striking that there is no ventilation during the lunch break, resulting in an increase in ambient temperature in the vehicle. It is hypothesized that due to this increase in ambient temperature pigs require a higher (air) circulation level i.e., higher heartbeat, to keep the body temperature constant.
Based on heart rate variability (HRV) data we did not find an effect of loading density during these transport trials. Differences in HRV were found between legs (periods during transport), and between the upper and lower floors (vertical placement). However, these differences in the HRV parameters are inconsistent and do not provide additional information to support assumptions based on other studies concerning physiological behaviour or stress.

4.4 Blood parameters:

It has been indicated in earlier work (Averós et al, 2009, Calà et al., 2009, Knowles and Warriss, 2007), that stress during transportation can cause an increase in blood levels of cortisol (during short journeys; Averós et al, 2007) (Cort) and glucose (Glu) concentrations and changes in white blood cell (WBC) counts. In the data presented in this report we found no significant changes in blood values related to stress. However, the P-value for the variation in cortisol (0.10) levels between low and normal loading density are interesting. During transportation at low loading density cortisol levels on average decreased by 6.2 ng/ml (from 41.3 – 34.6 ng/ml) whereas during normal loading density cortisol levels increased by 12.8ng/ml (37.0-49.1 ng/ml). Based on the average value and taking into account the large between-animal effects it is impossible to make a definitive conclusion on the relationship between loading density and stress.
Fatigue can be detected in changes in muscular intercellular enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH). Changes in aspartate aminotransferase (AST) during transportation have in the past been seen to indicate muscular degeneration resulting from road transportation. Low loading densities resulted in a close to significant lower level of CK and AST in the blood compared to the normal loading density. Taking into account the longer duration of fighting and the shorter period of resting in animals transported at the normal density it is considered reasonable that pigs transported at higher loading densities are more fatigued than animals transported at lower densities.
In retrospect the cortisol levels that were higher at normal densities compared to CK and AST levels and the shorter resting periods it can be argued that it is highly possible that pigs transported at normal density were more stressed than those transported at the lower loading density.
Increases in blood albumin and total protein concentrations can indicate an onset of the dehydration process (Averós et al, 2009, Averós et al., 2007,) in the body along with haematocrit (Ht) and red blood cell (RBC) counts. All animals had access to drinking water throughout the journey and average water usage per individual animal was 1 litre irrespective of loading density. Based on water usage, dehydration was not expected. Furthermore, blood protein and albumin levels as well as hematocrit and WBC values remained within normal ranges for all groups.

Conclusions

Mechanical ventilation fans were not used during the journeys or during the resting periods. This caused an increase in ambient temperature inside the vehicle. During more extreme weather conditions than experienced within this project it is very likely that lack of ventilation, particularly during stops, will compromise animal welfare .

Water usage per animal was similar irrespective loading density. During transport pigs use the drinkers and are willing to drink at the start of the journey.

Heart rate and body temperature were increased during handling and loading clearly indicating that this is a stressful part of transportation.

During the break almost all animals were resting at the same time. It is however striking that at lower loading densities a large number of pigs are already laying down during travelling while at higher loading densities almost no animals were laying down during travelling.
It is concluded that if pigs have enough space they will lay down for resting during driving often within 3-4 hours after departure.

Incidence of fighting bouts were influenced by loading density, period within the transport and formation of travelling groups by mixing unfamiliar animals.

The differences in cortisol, creatine kinase and aspartate aminotransferase were not significant but are indicative of fatigue due to increased activity or less resting and more fighting at the normal loading density.

In general, there were differences observed between journeys and between loading densities but due to the large differences between individual journeys, a sustainable conclusion on animal welfare in relation to stocking density can’t be made.
However, the differences in behavioural responses of pigs during transport, i.e., (fighting and resting), in heart rate levels and in blood values, tend to indicate that pigs can adapt more adequately to transport conditions when allowed more living space.

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