Animal Transport and Welfare with special emphasis on Transport time and Vibration

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Abstract

Farm animals are transported several times during their lifetime and often long distances, and most of them are to slaughter. During transport they are exposed to a number of stress inducing factors. The main objective of the thesis work was to study the welfare of pigs, cows and bulls during transport from farm to abattoir. Parameters such as cortisol, glucose, lactate, creatine kinease, behaviour, carcass pH, temperature, relative humidity, THI and vibration were used to evaluate welfare of the animals. Transport-related activities such as loading, transport, stopping, queuing, unloading and waiting at lairage and their durations were also monitored. Moreover locations of collection points and trucks routes were registered. During the experiments, GPS, temperature-relative humidity and vibration sensors and video camera were mounted on animals transport vehicles. The vehicles had natural ventilation and air suspension systems. Blood parameters and their correlation with transport time were evaluated. Cortisol concentration was negatively correlated in pigs, cows and bulls except for bulls at 12 h during summer. Correlation of lactate was strong and of glucose weak in pigs and cows. In bulls there were strong glucose (summer) and weak lactate correlations. Change in creatine kinease concentration in cows, bulls and pigs were positively correlated. However it increased at earlier transport time in cattle than in pigs. Animals behaviour such as lying, travel sickness (pigs), swaying and loss of balance (cattle) were strongly correlated with transport time. Transport of cattle on gravel road at 70 km h^{-1} induced highest vibration level, 2.27 m s^{-2}. Above 85% of the pigs in the third floor preferred lying in travel direction due to higher lateral acceleration. Pigs were exposed to heat and cattle to cold stress. Queuing at abattoir occurred in around 20 % of the deliveries. Queuing time at the abattoir varied between 7 and 98, with an average of 23.7 minutes. Potential savings for routes was up to 23%, therefore reducing negative impact on animal welfare and consequently on meat quality and environment. Pigs in the third floor were exposed to higher lateral acceleration. Based on behaviours and stress hormones, it could be concluded that on cattle an increase from 4 to 8 h and on pigs from 8 to 12 h transport time had higher effect on welfare. To reduce impact of vibration transporters have to adapt speed of vehicle to road and animals conditions.

Keywords: stress hormones, behaviour, optimisation, animal welfare, vibration

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Dedication

To Rahel and Anna

Science is organized knowledge. Wisdom is organized life.
Immanuel Kant

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ljungberg, D., Gebresenbet, G. and Aradom, S. (2007). Logistics Chain of Animal Transport and Abattoir Operations. *Biosystems Engineering* 96(2), 267–277.

II Gebresenbet, G., Aradom, S. Bulitta, F. S. and. Hjerpe, E. (2011). Vibration levels and frequencies on vehicle and animals during transport. *Biosystem Engineering* 110(1), 10-19.

III Aradom, S. and Gebresenbet, G. (2013). Vibration on Animal Transport Vehicles and Related Animal Behaviours with Special Focus on Pigs. *Journal of Agricultural Science and Technology* A (3), 231-245.

IV Aradom, S. Gebresenbet, G. Bulitta, F. S., Bobobee, E.Y.and Adam. M. (2012). Effect of Transport Times on Welfare of Pigs. *Journal of Agricultural Science and Technology* A (2), 544-562.

V Bulitta, F. S., Aradom, S. and Gebresenbet G. (2013). Effect of Transport Times of up to 12 Hours on Welfare of Cows and Bulls (publishing in progress).

All papers are reproduced with the permission of the publishers.

The contribution of Samuel Aradom to the papers included in this thesis was as follows:

Paper I: Data collection and writing.

Paper II: Data analysis, evaluation as well as manuscript writing.

Paper III: Data data collection, evaluation and writing.

Paper IV: Data collection, evaluating and writing.

Paper V: Data collection, evaluating and writing.

Publications not included in the thesis

I. Gebresenbet G., Bosona, T.G., Ljungberg, D., and Aradom, S. (2011). Optimisation analysis of large and small-scale abattoirs in relation to animal transport and meat distribution. *Australian Journal of Agricultural Engineering* 2(2), 31-39.

II. Ljungberg, D., Gebresenbet, G., Nordmark, I., Bosona, T. G., Aradom S., Jüriado, R. Cardoso M. and Redman L. (2012). Local food logistics, Report 055 (in Swedish). *Swedish University of Agricultural Sciences, Department of Energy and Technology.*

III. Bosona, T, Gebresenbet, G., Nordmark, I., Aradom, S. Ljungberg, D. (2011). Box-scheme based delivery system of locally produced organic food: Evaluation of logistics performance. *Journal of Service Science and Management* 4(3), P357-367.

IV. Aradom, S., Ahlgren, S., and Sundberg, C. (2013). Current situation of biofuels development in Sub-Saharan Africa – policy, production and research. Report No 2013:11, f3 *The Swedish Knowledge Centre for Renewable Transportation Fuels and Foundation, Sweden.*

Abbreviations

a	Acceleration, m s^{-2}
C	Cortisol
CF	Crest factor
Ck	Creatine kinease
DFD	Dark firm and dry
eVDV	Estimated vibration dose value, m $s^{-1.75}$
FF	Forward orientation
Ft	Fighting
G	Glucose
L	Lactate
Ln	Lying one on another
Ls	Loss of balance
Ly	Lying
PP	Perpendicular orientation
PSD	Power spectral density,
PSE	Pale soft and exudative
Pt	Panting
R^2	Coifficient of determination
R	Correlation of coifficient
Rc	Restlessness and change of position
Rd	Refuse to go down the ramp
r.m.s	Root mean square, m s^{-2}
Rt	Rooting
SCAHAW	Scientific Committee on Animal Health and Animal Welfare
St	Sitting
T	Transport time
THI	Thermal-humidity index
Vc	Vocalisation
VDV	Vibration dose value, m $s^{-1.75}$

1 Introduction

1.1 Background

Today, modern farm moves towards large-scale intensification in farm animals keeping methods have meant that on big farms large numbers of animal are looked after by small number of attendants. Many of the regular stock tasks are carried out by machines. As a result of the low need for close regular physical contact between human and cattle, there has perhaps been a tolerance of uncooperative behaviour and less incentive to select for quietness in the stock (Ewbank, 2000). The considerable increase in number of animals in farms has led to the rise of transport. According to Harris (2001), transport is a large and integral part of today's livestock industry. Transport and handling of farm animals comprise multiple of activities at all levels in farms, during transport and at abattoir operations chains and all are stressful to animals. Logistics chain of farm animals in contrast to physical distributions of goods is the process of collecting animals from various points of origin and moving them to a single destination. Therefore they are usually loaded from single or several farms in one vehicle and transported to one destination point. Some of the stressful activities are loading and unloading procedures. They involve separation of the animals from familiar groups and original places and eventually move to new areas where they are subjected to restricted space, mixing with unfamiliar animals, noise, vibrations and microclimatic environment etc. Moreover level of handling methods and facilities of getting the animals on to, out of the truck and subsequent queuing at the abattoir and operations at the slaughter house have strong influence to ease or worsen the stressful situation. Effective logistics and dynamic planning process that take into account size of holding pens, animal species, geographical locations, seasonality, road and traffic conditions can provide reduced stress (shorter distance and time), environmental benefit and low operational cost. Ride comfort which is also influenced by design of a vehicle, road condition and driving performance are stress inducing

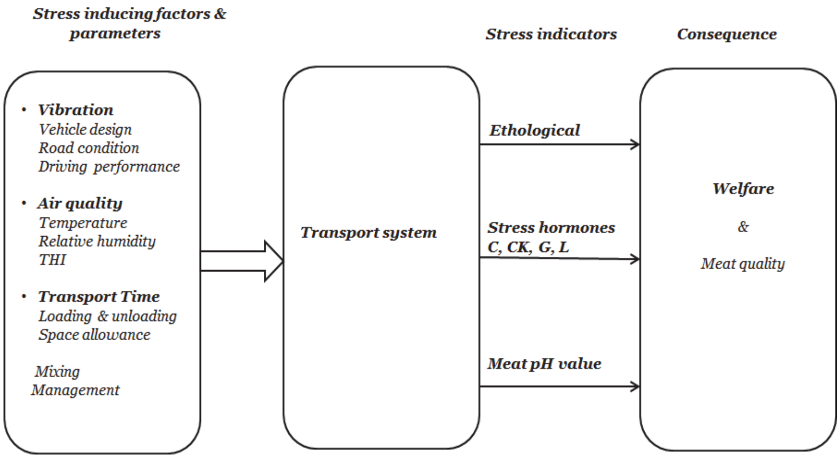


Figure 1. A chart showing stress inducing and indicators during transport and their consequences.

factors. These factors combined with temperature, relative humidity, length of transport time and other transport conditions impose stress on the animals and compromise their welfare and are indicated in Figure 1. Some of the main stress inducing factors such as vibration, temperature and relative humidity were measured. Furthermore, stress indicating parameters blood hormones; behaviours and carcass pH value were measured to evaluate welfare of the animals. Stress inducing factors can be minimized by improving animal transport logistic system and handling methods that include optimized abattoir activities etc (Gebresenbet, 2003). This could also results in a good welfare, improved meat quality and reduced environmental impact and cost.

1.2 Literature review

Farm animals and transport

Farm animals are transported several times during their lifetime, and most are transported to slaughter, often over long distances, both within and between countries (Appleby, 2011) and it is an exceptionally stressful as it compromises their welfare (Knowles & Warriss, 2000). Every year around 45 million cattle (cows, beef cattle and calves) are transported around EU for the purpose of breeding, fattening, and slaughter and 255 million pigs were transported and slaughtered in 2008.The biggest trade is in the road transport to slaughter (Marquer, 2010; EU, 2013). Partly as a result of this intensification there has been increasing concern about the welfare of farm animals (Appleby, 2011). According to Duncan and Fraser (1997) animal welfare arose in society to express ethical concerns regarding their treatment. Transport and the associated handling may also cause important losses to the livestock industry (Grandin, 2000). Social concern about the consequences of transport on animal welfare has gradually increased during recent decades resulting in a legislation being past in the European Union (Council Regulation 1/2005/EC). Today animals are defined as “sentient creatures” in European law and no longer just as agricultural products (Treaty of Amsterdam, 1997). That change reflects ethical public concern about the quality of life of the animals (European Food Safety Authority, 2004). The review examines the input of effective logistics chain and abattoir operation on welfare of animals, meat quality and environmental benefits. The literature review also included the effect of transport time and vibration on farm animals in terms of welfare and the main tools employed to assess welfare are considered in detailed.

Logistics chain of animal transport and abattoir operations

Logistics chain of farm animals and abattoir operations and some of the implications to the animals are reviewed below. In intensive production system, lambs were loaded at the farm, unloaded at classification centres, weighed, classified and distributed to feed lot pens until they reach slaughter weight. This process is a source of new stressors including social stress due to mixing. Thus extra handling procedures in pre-slaughter logistic chain often exacerbate these effects and reduce welfare of the animals (Miranda-de la Lama et al., 2008). Lambs were transported from the original farm to temporary residence. Depending upon their weight they stay 7 or 28 days or reloaded immediately for slaughter. It was concluded that stay time had significant effect ($P<0.001$) on meat texture (Miranda-de la Lama et al., 2009). A small scale local abattoir that depends on regional supply of animals would have direct impact on animals welfare, meat quality and environment. Reductions in transport time and distance during collecting animals from farms and to abattoir were 42% and 37% respectively. The corresponding greenhouse gas (GHG) emissions generated from vehicles reduced by 42%. Moreover consumers in the region strongly preferred locally produced meat (Gebresenbet et al., 2011). According to Meenach et al., (2005), processing plants of livestock are close to livestock production locations in the state of Washington. This has resulted shipment destination of 78.16% of the livestock be within the state. It is also more efficient packing and transport of processed meat when compared to live animals. Rijpkema (2011) reported that European pork supply chains consist of production and processing systems including marketing segments. Despite the recent development of sensory technology and ICT (Information and Communication Technologies) the author concluded that the

logistics processes are still subject to a number of inefficiencies, like operational inefficiencies or low product quality. This results in suboptimal market performance and an increase of environmental impact.
In a prototype web-based geographic information system, vehicle geographic coordinates, date, time, and temperature etc. were recorded. The system enabled immediate visualization on map of the current position of animal being transported and also makes it possible to monitor the status of animal welfare (Ippoliti et al., 2007).
A slaughter house with limited holding-pen space it is important to schedule truck arrivals precisely to assure a continuous supply and animal welfare (Gregory, 1998). Time window of animals to be delivered to abattoir for slaughter is variable depending upon the species. A pig ready for slaughter has about a five day interval where slaughter can be done before it gets too heavy. For healthy cattle, the interval is about three weeks (Wiberg et al., 2007). The time window of various animals can therefore be effectively utilized in a flexible manner for planning and optimizing the routes.
Logistics chain of animals transport to abattoir is a complex task and has specific characteristics. Transporters collect the animals from various farms to a single abattoir. In respect to animal welfare, distance and time have to be minimised by choosing the best possible route. To ensure elimination or reduction of early or late arrival of vehicles at the abattoir, transport planning should not focus only on continuous supply of slaughter animals to the abattoir.

Vibration

Vibration is a term that describes oscillation in a mechanical system. It is either the motion of a physical object or structure or, alternatively, an oscillating force applied to a mechanical system (Harris & Piersol 2010). Vibration is characterized by its direction (travel of direction, vertical and lateral) and amount of frequency and acceleration. The frequency of a vibration influences the extents to which vibration is transmitted to the surface of the body or through the body (Griffin, 2012). During transport of farm animals, vibration is one of the potential stressors. Vibration, which depends on vehicle design, and the jolting, shocks, and sudden impacts caused by road conditions and driving skill, may compromise animal welfare (Peeters et al. 2008). A large, fixed-body truck with air suspension provides a very smooth ride, classified as not uncomfortable to a little uncomfortable (Randall et al., 1996). Few vibration studies were carried out on poultry, pigs and cattle. On a vehicle used for poultry transport, Scott (1994) reported that, the fundamental frequency is 1-2 Hz and with secondary peak occurring at 10 Hz, the value coincides with the resonance frequency of poultry viscera. Detailed measurements by Randall et al., (1996) found that resonance frequency of broilers were around 15 Hz when sitting and 4 Hz when standing. Warriss et al., (1997) reported broiler chickens subjected to narrow band vibration (2, 5 and 10 Hz) at 2 ms^{-2} root mean square, had decreased pH ultimate in both pectoralis superficialis (PS) and biceps femoris (BF) muscles. Furthermore as the frequency increased there appeared to be greater depletion of glycogen in the liver. Pigs with a body weight 20 and 25 kg were vibrated in a vertical direction for 2, 4, 8 and 18 Hz in combination with root mean square 1 or 3 ms^{-2}. Comparing with 2 and 4 Hz spent time lying was 10 times shorter at 8 Hz and 18 times shorter at 18 Hz (Perremans et al., 2001). The proportion of pigs standing or lying was associated with an increase or decrease in acceleration (Peeters et al., 2008). According to Stevens and Camp (1979) and Singh (1991) vibration of transport vehicle is believed to be one of the stressors linked to development of shipping fever in calves. The authors have investigated the location on the trailer which provided the most severe shock and vibration inputs to the cattle; both of the studies concluded that cattle located over the rear axles of the livestock trailer are subjected to the greatest vibrations and recommended lower inflation of tires and air ride rather than leaf spring suspension. Calves subjected to vibrations at a frequency of 2 Hz become more stressed compared with vibrations of 12 Hz (Van de Water et al., 2003 a, b). Wikner et al., (2003) investigated vibration on cattle transport truck and found that most of the animals change position in perpendicular orientation to the vehicle's motion. The authors further reported that vibration values are higher on gravel than paved road surface for unloaded cattle transport vehicle. The adverse effect of vibration on animals is not limited only during transport but also common in dairy farms. According to Nosal and Gygax (2005), when noise (> 70 dB) and vibration (> 0.3 ms^{-2}) of milking parlour is beyond the desired values, it adversely affects udder health and the welfare and performance of both cow and milker. Unlike driver or passenger, animals are transported on vehicle's container. Road conditions, vehicle speed, driving style and standing orientation of the animals have significant influence on vibration levels. On a livestock transporter, the interfaces between the animals and the vehicle are not designed to reduce vibration and it is possible that animals are subjected to a higher vibration magnitude than the driver (Randall, 1992). Vibration on animals has received little attention despite the recognition that in human and animals it may induce fear, nausea, distress and fatigue because of the distribution of the oscillatory motions and forces within the body (Randall et al., 1993). Today multi-floors, semi-trailer trucks are widely used for pigs transport. Owing to vehicle design, it is not known whether pigs in all the floors are exposed to the same level of vibration. In respect to welfare, health and performance, there is lack of pigs and cattle vibration data which limits determining the comfort ranges of vibration levels.

Temperature and relative humidity

The field experiment was conducted (winter and summer) in 2008 in Sweden where temperature varied widely during hot and cold seasons. Cattle and pigs were transported in vehicles with natural ventilation systems that entirely relied upon the movement of a vehicle. In natural ventilated vehicles microclimatic development has important implication for welfare of farm animals. When a vehicle stops for loading and other activities sudden rise of ambient temperature and relative humidity can occur. The case may be critical during hot seasons. Review on some of the main impacts of hot and cold weather conditions and length of transport on both cattle and pigs are summarized below.

In a natural ventilation trailer the environments rely on the openings of the trailer, wind and movement (Burlinguette et al., 2011). High temperatures and high humidity prevent the animals releasing their heat. Low temperatures and high air humidity may enhance heat dissipation and the animals suffer from cold, but the worst conditions for heat loss occur at high temperatures and high humidity (EC, 1999). During transport of animals on lorries the main criterion is to prevent the Upper Crtical Temperature (UCT) from being exceeded. An air temperature of 32°C could be selected as a maximum design value. When ambient temperature changes animals use both physiological and behavioural mechanisms either to increase heat production or to promote heat loss and maintain homeothermic (National Research Council Committee, 2006).

The performance, health, and well-being of cattle are strongly affected by climate. During warm weather, cattle show signs of stressful behaviour, impaired physiological functions and increased incidence of morbidity (Hahn & Mader, 1997). They are considered more sensitive to hot than to cool temperatures (Baker et al. 1981). According to Holleben et al., (2003) in cattle transport vehicle the average inside temperature was 3 ^{0}C higher than outside. The inside ranged from 0.3 to 22 ^{0}C while outside temperature ranged from -4 to 19 ^{0}C. The likelihood of cattle death increased sharply when the midpoint ambient temperature fell below −15 ºC and becoming non-ambulatory increased when temperatures rose above 30 ºC (González et al., 2012d). In another study to identify and quantify factors affecting shrink in cattle during commercial long haul ($\geq$ 400 km) the same authors reported that shrink increased with both midpoint ambient temperature (% of BW/ºC) and time on truck (% of BW/h). Furthermore they found that temperature and time on truck had a multiplicative effect on each other. In cattle shrink increased most rapidly for both longer durations and at higher ambient temperatures (González et al., 2012a).

In pigs transport truck, Sällvik et al. (2004) further reported that the temperature inside a standing vehicle with natural ventilation at loading increased by 0.15 ^{0}C/min. Pigs cannot cool their skin by transpiration because their sweat glands are very few and their distribution is confined almost entirely to the snout (Ekesbo, 2011). The author further states that when weather is hot pigs seek places to wet themselves thus relying on wallowing for cooling. However, wallowing or lying on moist places is impractical on transport truck. Sällvik et al., (2004) further reported that, the number of pigs which showed panting increased when the temperatures of transport vehicle exceeded 25 ^{0}C. Investigation on mortality rate was carried out during transport on 10.3 million pigs. The rate increased from 0.07% for outside temperatures lower than 5°C to 0.11% for temperatures higher than 15°C. It also increased with the length of the journey from 0.084% for journeys shorter than 75km to 0.12% for journeys longer than 150 km (Colleu & Chevillon, 1999). Honkavaara (1989) also investigated pigs' mortality during winter and summer conditions in Finland. It was low during heavy frost (-30 to –20 °C), increased considerably in cold (-3 to 0 ^{0}C) and was highest in warm weather (16 to 26 ^{0}C). Similar results are also reported by other authors that losses to be higher (up to 0.4%) when the environmental and internal trailer temperatures increase in summer months (Warriss & Brown, 1994; Haley et al., 2008a; Haley et al., 2008b).

Small rises from the normal pig body temperature of 39–42 degrees proved to be fatal (Lambooij & van Putten 1993), so adequate ventilation on transport vehicles and the weather of the day must be taken into consideration.

Thermal stress is recognised as one of the major reasons of reduced welfare and health of the animals which can affect end product quality and may even cause death (Dantzer, 1982). Thus to prevent poor welfare, fluctuations of temperature and relative humidity should be within the optimum comfort range of the animals in transit.

The Upper Critical Temperature (UCT) for finishing pigs may be as low as 23°C, depending on factors such as body size, feeding, and hydration status, and conditions of transport such as load density (Lambooij, 2000; Randall, 1993; Schrama, 1996). No signs of thermal stress were observed in slaughter pigs on transport vehicles at air temperatures between 10 and 25 ^{0}C in France (Chevillon *et al.,* 2002). In Canada the recommended thermal comfort zone for market weight pigs is 10°C to 21°C (Agriculture & Agri-Food Canada, 1993).

Unfavorable climatic condition inside cattle and pigs transport vehicles have been reviewed and some of the main welfare impacts are: dehydration, fatigue, lame, non- ambulatory, body weight loss and death. Moreover the interaction of climatic condition and length of transport adversely affects welfare of the animals. There are several studies on hot temperatures and high relative humidity and their negative effects on animals in transit. Nonetheless little data is available on the combined effect of declining temperature and rising relative humidity. Mainly when temperature goes below the recommended level.

Effect of transport time on welfare of cattle and pigs

Several studies have been conducted regarding the effect of transport time or distance on welfare of cattle and pigs and the main welfare effects are given below.

In modern agriculture, animals are exposed to long distance transport, comparatively novel and, too often their coping strategies are over challenged.

In the last few years, the centralization of slaughter industry, with more animals being killed in fewer large plants, has modified the distance that animals must be transported to the slaughterhouse (Bench, 2007; Bench et al., 2008).

Gebresenbet et al. (2005) transported calves, young bulls and cows for short (≤ 1 h), medium (4-6 h) and long (8-11 h) transport time to investigate effect of duration on cattle welfare and post mortem meat quality. They concluded that transport time after six h is particularly stressful for the animals when transported with usual vehicles without special equipment. However, less detrimental effect of transport time on meat quality has been observed.

Warriss et al. (1995) transported steers for five, 10, or 15 h at distances of 286, 530, and 738 km, respectively and lost 4.6, 6.5 and 7% of their bodyweight as journey time increased. The authors also found that increased duration led to dehydration and fatigue. However they concluded that when transport conditions are good, 15 h transport is not detrimental to cattle welfare. Gallo et al. (2003) also transported slaughter steers for three or 16 h and it was found that the longer journey was associated with a significantly larger live weight loss. In long distance road transport of heifer, bulls and steers, it was reported that loss of body weight in steers (6.65%) coming from pasture was higher compared to bulls (4.6%) (Marahrens et al., 2003).

A study was performed to investigate the relationship between long distance (≥400 km) transport and welfare on 290,866 animals (6,152 journeys). Time spent on the truck, loading time, animal density and season were some of the surveyed transport conditions. Overall 0.012% of assessed animals became lame (difficult to walk), 0.022% non-ambulatory (not able to walk about) and 0.011% died on board. The likelihood of cattle becoming non-ambulatory, lame, or dead increased sharply after animals spent over 30 h on truck. Likewise animals that lost 10% of their body weight during transport had a greater likelihood of dying and becoming non-ambulatory or lame (González et al., 2012). Regarding cattle transport, several North America studies have documented that transport durations ranging between 2 and 48 h result in shrink values between 0 and 8% of body weight (Jones et al., 1990; Schwartzkopf-Genswein et al., 2006).

Various pigs' transport studies have reported that rate of dehydration is greatly accelerated by road transportation (Becker *et al*., 1989). Increasing transport duration increased drinking post-transport and blood haematocrit, which is an indicative of rising levels of dehydration and thirst (Lewis & McGlone, 2007). But some researchers did not make emphasis on journey time only they also included loading and unloading durations and transport conditions as well. Ritter et al. (2006) found that total time from loading to unloading was positively correlated with transport losses (dead and non-ambulatory pigs). The mixing of unfamiliar pigs at loading can increase both transport deaths and carcass damage (Gosalvez et al., 2006). Bradshaw *et al*. (1996b) also reported increased cortisol levels, on 1.5 and 8 h journey time and led them to the conclusion that this rise, initially a response to loading, was maintained as a

response to transport. According to Lambooij (1998), the range of weight loss in pigs, even in short-term transport is between 4-6%. It has also been reported that not only long transport time (8 h), even short (1 h) journeys can affect the welfare of the animals with increased mortalities and pathological findings (Werner et al., 2007). Mota-Rojas et al. (2006) exposed fattening pigs for eight, 16 or 24 h transport without access to food and water. They reported incidence of bruising, redness of the skin, shaky-leg syndrome and number of pigs lying down upon arrival all increased with increasing journey duration. Furthermore, weight loss was 2.7%, 4.3% and 6.8%, during eight, l6 and 24 h transport time respectively. However, eight h of lairage allowed the pigs to regain some weight due to rehydration. In a similar study, Brown et al. (1999) transported pigs (80 to 100 kg) for 0, 8, 16 or 24 h, and found a significant increase in plasma lactate and cortisol concentrations following 16 h of transport. Weight loss, as well as plasma protein and albumin concentrations increased with increasing transport duration indicating dehydration. Without specifying transport conditions, data collected through 10 years of pig slaughtering in the Czech Republic, Malena et al. (2007) reported that mortality in fattened pigs increased with distance travelled (0.07% for journeys under 50 km and 0.32% for journeys over 300 km). On the basis of data collected on 2.7 million pigs in the USA, Southerland et al., (2009) found that the effect of journey time on the percentage of dead pigs increased for journeys lasting more than 30 minutes and decreased for journeys lasting between 5 and 11 h and the authors concluded that, mortality didn't increase in proportion of travelled time. According to Gosálvez et al. (2006) based on the data from 496 journeys of more than 90 000 slaughter pigs, they found that mortality increased with transport distance, as did weight loss. An interaction was also found between transport distance and number of farms from which the pigs were collected. Mortality was 0.2% to 0.3% when pigs were transported from a single location. However, mortality increased to 0.7% when pigs from several farms were involved and the distance was over 100 km.
Weight loss attributable to withdrawal of food and water during journeys represents an economic loss (Lambooij, 2007). Among other factors, it is known that the duration of the journey has a negative influence on welfare and meat quality (Bradshaw et al., 1996; Perez et al., 2002; Mota-Rojas et al., 2006). Mortaltity is an evident indicator of poor welfare during transport (Warriss & Brown, 1994; Warriss, 1996a). The literature review of transport time and its impact on welfare of cattle and pigs provides broad understanding but there are certain points that require consideration. A number of investigations have shown that, loading and unloading give rise to a stressful situation. Although they remain the main components of transport, without which transport cannot be achieved regardless the duration or distance. But during studying duration or distance of transport, sufficient emphasis is not given to effect of loading and unloading and the way they are performed. Some authors measure transport in distance (km) and others in time (h) to relate its impact on welfare of cattle and pigs. When transport is expressed in

terms of distance it remains unclear the length of duration the animals spent in the trucks. When loadings take place from various farms but in one vehicle, distances and time between collection points and destination cannot provide the same effect on welfare. It is also important to note that, suppose animals enter the vehicle in a very stressed mode and combined with the new environment in the vehicle this can make situation of the animals more severe. According to Grandin, (2008) a 500 km trip on a smooth highway will probably be less stressful than a 100 km trip on a bumpy dirty road that takes the same length of time. It should be important to detect conditions under which loading, transport and unloading of animals are performed. When proper handling is provided before and throughout transport duration, stress levels may be reduced even during long journeys (Brown et al., 1999). The condition under which animals are transported, rather that the act of transportation per se, may induce marked physiological stress which in turn, will compromise their welfare in transit (Malcom & Peter, 2008). During long duration of transport some authors did not provide detailed accounts as related to ventilation systems (natural or forced) of the trucks. It remains unclear when transporters of naturally ventilated trucks take rest and trucks are not moving. In situation like this, there is rapid rise of temperature and relative humidity which affects welfare of the animals.

Welfare assessment during transport

Welfare of an animal is its state as regards its attempts to cope with its environment Broom (1986). Fraser (1993) refers to well-being as the state of the animal and assessing it in terms of the level of biological functioning such as injury or malnutrition, extent of suffering and of positive experience. Dantzer (1982) identified many welfare problems related to transport of farm animals. Most of them could be solved by application of the existing legistlation and better co-ordination, although there still remained a need for more information on vehicle designs, stocking densities, animals handling conditions and other factors relative to the knowledge of physiological and behavioural needs of farm animals. Welfare can be measured in a scientific way that is independent of moral considerations (Faucitano & Schaefer, 2008). However it cannot be measured directly, but the consequences of different causes of suffering and satisfaction can be compared in various ways (Lester *et al.,* 1996). Studies to assess animal welfare during handling and transport should contain both behavioural and physiological measurements (Grandin, 1997). The main parameters considered when assessing animal welfare are: stress hormones, behavioural alteration, meat pH value, temperature, relative humidity and vibration.

Stress hormones

Stress can be defined as an environmental effect on an individual animal which overtaxes its control systems and reduced its fitness or seems likely to do so (Broom, 2001a; Broom, 2007). It therefore refers to the coping of the animal with the environment, describing the animal's state when it is challenged beyond its behavioural and physiological capacities to adapt to its environment (Terlouw et al., 1997). Stress is a norm in social animals and may or may not be damaging (Siegel & Gross, 2000). Whether a stressor can be considered as harmful depends on the way an organism is able to cope with a threatening situation as it regains a state of homeostasis (von Borell, 2000 & 2001). Behavioural and physiological changes related to the stress response can yield very useful information (Fraser & Broom, 1990; Warriss *et al*., 1998b). The stress associated with transport and arrival of newly received cattle at a new facility can contribute to physiological changes including transient changes in endocrine hormones, altered metabolic enzymes and metabolites associated with energy and protein metabolism (glucose, creatine phosphokinase, and lactate dehydrogenase, etc) (Loerch & Fluharty, 1999).
Plasma measures, such as creatine kinase (CK), lactate dehydrogenase (LDH) and cortisol, are reported to change in response to stressors (Helmreich et al., 2006, Jarvis et al., 2006), and are often used to reflect stress coping characteristics and metabolic status of the animal. Knowles and Warriss (2000), also identified main causes and physiological indicators of stresses during transport like, fear or arousal increases cortisol concentration, physical exertion elevates creatine kinease and lactate levels and motion sickness raises vasopressin concentration in the blood. The concentration of cortisol in blood is widely used as an indicator of stress, although caution is advised, since an increase does not occur with every type of stressor (Terlouw et al., 1997). According to Moberg (1985) individual animals of the same species may handle threatening situation differently. Studies to determine the amount of stress of farm animals during transport often have highly variable results and are more difficult to interpret from an animal welfare standpoint (Grandin, 1997). Schrader and Todt (1998) reported that in pigs, peripheral endocrine stress responses are accompanied by changing rates of specific types of vocalizations. Animal stress response expressed in blood hormone concentration level, it is usually compared to the base line value because thresh hold is lacking to indicate the degree of stress. Hormones responses to handling and transport can vary considerably from one animal to another even in the same farm; therefore it should be combined with behavioural studies to acquire a better picture of animal welfare. Measurement of animal welfare is not always easy and several welfare indicators should be used (Grandin, 2000).

Stress and behavioural changes of animals

Animal behaviour has been defined as the overt and composite functioning of the animals individually or collectively and it is the means whereby the animal mediates dynamically with its environment both animate and inanimate (Fraser, 1980) and like physiology and anatomy, behaviour is part of the general functioning of an animal (Broom, 1981). According to Ekesbo (2011) it is the basis for ascertaining animal welfare. It is also non-invasive and in many cases non-intrusive (Dawkins (2004). The most obvious indicators that an animal is having difficulty coping with handling and transport are changes in behaviour, which show that some aspect of the situation is aversive (Broom, 2000). Behavioural changes can be quantified using comparisons of responses (stopping when they encounter shadows, bright areas, dark areas, etc.) Grandin (1981a, 1997, 1998). While it may intuitively appear that fearful animals may be easy to move, animals that are fearful of humans are likely to be the most difficult to handle (Hemsworth, 2007). To measure severity of stress during transport, behavioural indicators have been usually used. An important behavioural measure of welfare when animals are transported is the amount of fighting which they show; the recording of such behaviour should include the occurrence of threats as well as the contact behaviours which might cause injury (Broom, 2000). Pigs and cattle are social animals and they are affected by separation and mixing during transport. But they may not depict stress related behaviours in exactly the same manner. Behavioural responses of farm animals to stressing situations are species based. Several studies have shown that pigs significantly increase their non-vocal and vocal behavioural activities in stressful situations, such as in social separation (Fraser 1974 & 1975). They try to stay in contact with one another in stressful conditions and this can be seen in huddling behaviour during transport as they are calmed by the presence of their pen mates (Lambooij & van Putten 1993). Randall, (1992) reported that pigs are affected by motion sickness during transport. Warriss et al. (1998b) investigated skin-blemish damage in 63% of slaughter pigs (10% had moderate damage) and concluded that fighting between mixed groups of unfamiliar animals was the probable cause. When deprived of feed for approximately one hour, 12 hours or 18 hours, Brown *et al*. (1999b) found that pigs deprived of feed for 18 hours engaged in more fighting during the lairage period than those pigs only deprived feed for up to an hour. There may need to differentiate loading densities for long and short journeys. On longer journeys, pigs welfare will be severely compromised if they are unable to lie down (Warriss et al., 1998b). When weather is hot pigs seek places to wet themselves thus relying on wallowing for cooling (Ekesbo, 2011). During hot temperature in a vehicle the animals are unable to express some specific behaviour like wallowing.

Cattle are generally anxious and restless and defecate and urinate frequently at the beginning of a journey (Knowles at al., 1999). During cattle transport Tarrant and Grandin (2000) reported that restlessness can result in changes in position triggered by social interactions, such as chin-resting and mounting, and also by driving events, particularly cornering. Furthermore they found that maintenance of balance depends most on driving events like braking, gear changing, cornering and slipperiness of the floor surfaces. But falling is the major risk and it is greatly increased at high stocking density and by driving events. Tarrant et al., (1992), investigated long (24 h) transport of steers and the effect of stocking density on physiology, behaviour and carcass quality. They found that most common standing orientation was perpendicular to the direction of travel and some animals lay down during transit at all stocking densities. But at high stocking density the animals were trapped down and unable to rise. According to Tarrant (1990) loss of balance is a major determinant in injuries in transported cattle. In the study the author found that one-third of events where cattle were floored during transport were caused by loss of balance during cornering. Knowles et al. (1999b) also studied cattle transport for 14, 21, 26 and 31 h including a stop for a rest and drink on the lorry after 14 h. Based on the physiological measurements the authors concluded that a journey lasting 31 h was not excessively physically demanding, but many of the animals chose to lie down after about 24 h. According to Jensen et al., (2005) in non- transport environment cattle prioritise lying down of approximately 13 h per 24 h. Furthermore, when cattle left undisturbed, they will spend approximately 12 h resting in recumbency every day (Munksgaard et al., 2005). But according to Eicher (2001) during transport, adult cattle stand more, but lie more during the recovery period. In relation to farm environment, cattle and pigs in transit are deprived of expressing some specific behaviours and absence of such behaviours have negative impact on welfare. Behaviour is a good means of assessing stress of farm animals when exposed to new or unfamiliar environment. Detailed behavioural information can be acquired through observation and without causing additional stress. But all the behaviours displayed may not indicate same level of stress. Method of assessing need to be developed to indicate severity of stress in a quantifiable way.

Meat pH level

Welfare measurement is not always easy and several welfare indicators need to be used in order to draw an accurate conclusion (Bench, 2007). Stress before slaughter can have impact on both welfare and end quality of meat. Acute stressor imposed on pigs immediately prior to slaughter will increase muscle temperature, increase lactic acid concentration, and increase rate of muscle pH decline post-slaughter (Moss, 1984), which in turn can lead to pale, soft and exudative (PSE) pork. Pérez *et al*. (2002) reported that pigs transported for only 15 minutes showed significantly lower pH values in various muscle groups compared with animals transported for 3 h. On the other extreme, prolonged stress prior to slaughter can lead to depleted muscle glycogen concentrations, low muscle lactic acid concentration post-slaughter, high ultimate pH, and dark, firm, dry (DFD) pork (Tarrant, 1989; McPhee & Trout 1995). According to Tatum et al., (2007) cattle subjected to pre-slaughter stress often produce carcasses with higher-than-normal muscle pH and darker-than-normal lean colour (dark cutter). The degree of the dark cutting condition is dependent upon final pH of the carcass musculature. Both Pale soft and exudative (PSE) and DFD meat cuase substantial economic losses especially dark cutting (Frase & Broom, 1997). The likelihood of DFD and PSE meat is related to pre-slaughter stress (Honkavaara, 1989; Gregory, 2007). Fàbrega et al. (2002) also found a relationship between a high percentage of dark, firm, and dry (DFD) meat and a lower welfare index. Stress in farm animals can have detrimental effects on the quality of food products (Rostagno, 2010). Thus, animal welfare and meat quality are inseparable. Although pH value of the carcass is within the normal range the animals may still be under stressful situation. Gruber et al., (2006) suggests that avoiding stress immediately prior to harvest is important for assuring acceptable beef tenderness, even if muscle pH remains unaffected. Therefore there is no consistent association between indices of stress and meat quality parameters (Warriss *et al*., 1998b; Bradshaw *et al*., 1999). Welfare of animals which may vary from poor to good can be assessed by evaluating stress hormones, ethological parameters, carcasses pH level, temperature, relative humidity and vibration levels.

2 Objectives and structure of the thesis

Objectives

The main objective of the thesis work was to study the welfare of farm animals during transport from farm to abattoir. The specific objectives were to investigate:

- logistics chain of animal transport and abattoir operations, and demonstrate the potential effect of operations planning and route optimization on animal welfare, meat quality, and environment (Paper I);
- vibration and frequency levels on typical cattle transport vehicle and animals (Paper II);
- transmissibility of vibration from floor 1 to floor 3 and driving performance of pigs transport vehicle (Paper III);
- effect of vibration on behaviour of pigs and on postural stability of cattle (Paper II and III) and
- effect of transport times of up to 12 h on the welfare of bulls, cows and pigs (Paper VI and V).

Structure of the thesis

The scope of the study is illustrated in Figure 2.The thesis is composed of five papers dealing with transport of farm animals from farms to abattoirs. Paper I studies the logistics chains of livestock (cattle, pigs and sheep) transport including certain operations at the abattoir. The evaluated parameters included location and duration of activities at farms level, during transport routes (including distances) and at the abattoir. Vibrations measured on animals transport vehicles and on animals as well as related behaviours are covered in papers II and III. Vibration is one of stress inducing factors and it was also evaluated in respect to related animals behaviour.

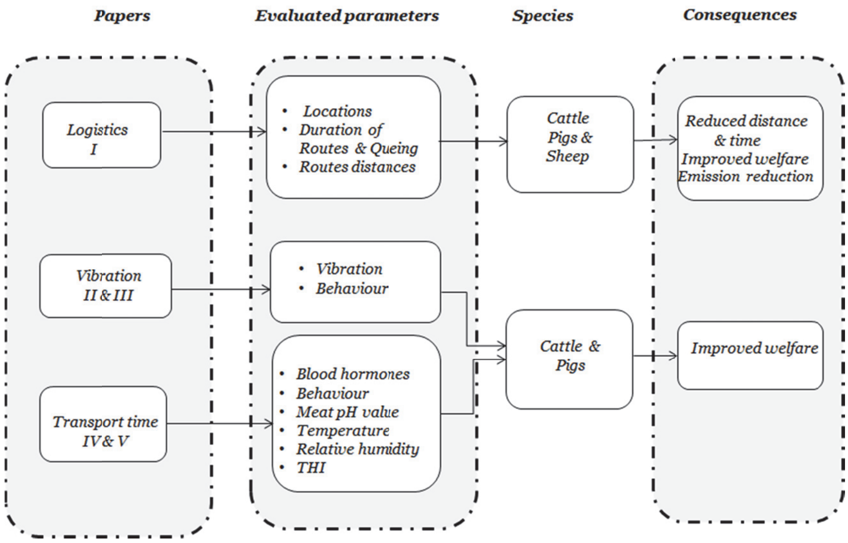


Figure 2. Thesis structure showing the evaluated parameters of animal welfare during transport.

Papers IV and V reported on transport time and its effect on the welfare of cows, bulls and pigs. Multiple parameters including blood hormones, behaviour, carcass pH value and climatic conditions were employed to evaluate welfare of transported animals. The result of the thesis work is intended to help improve welfare of transported animals by improving transport conditions, through effective planning and optimising routes not only from loading to unloading but up to the final stage of stunning.

3 Materials and Methods

The current studies were performed on farms, in transit and at abattoirs during transport of animals from farm to abattoirs. The studies consisted of field measurements, interviews, observations and activities documentation. Conventional and commercial livestock transporter vehicles were used for the field experiments.

3.1 Logistics chain of animal transport and abattoir operations

During collecting farm animals from various farms to the abattoir the main activities considered were loading, transporting, stopping, queuing (loaded and at vehicle washing) and unloading. The activity of slaughter animals was extended from the lairage box to stunning point and the abattoir chain of operations continued to carcasses cooling room.

Interviews, field measurements and instrumentation

Investigation based on interviews was carried out with drivers of cattle transport to obtain a general understanding on the patterns of animal transport routes. The information collected was later used to serve as a reference to the observed and measured data. Interviews were also conducted with key persons in the abattoir to acquire better understanding on logistics chain and planning for field measurement. Detailed data collection was made at three levels: (a) Transport route on-board activity registration: Time used for loading, driving, and unloading was registered. Geographic positions and all activities performed were documented using GPS receiver. Furthermore, geographic positions were registered during driving (reference points) to enable replication of the route choice in-between collection points. (b) Delivery gate activity registration (including vehicle and animal activities): The observed activities at the abattoir were classified as vehicle and animal activities.

Vehicle activities were based on observation of the vehicle or the load and all animals on the vehicle seen as one entity while animal activities were based on observations of individual animals. The activities included vehicle arrival and queuing, unloading, and washing and animals movement and lairage. In total there were 60 deliveries of farm animals that consisted of 31 cattle, 18 pigs and 1 sheep during 30 h registration. (c) Slaughter chain activity registration: Description of the slaughter chain of operations was mapped out in detailed based on the information collected through interviews and direct observations. Duration of observation was 20 h. The slaughter chain activities involved in the description was from stunning to carcass cooling room. Hand held GPS receiver was used to record time and geographic positions (Magellan GPS 315). Accuracy of measurement, as stated by the producer, was 15 m root mean square (RMS).

Analysis

The analysis of results concentrated on identification of bottlenecks in the chain. The registered data were analysed to determine the impact of frequency of arrival of animal on-board vehicles and duration of activities, queues, capacity utilization, and other system constraints. Route LogiX Professional v2.15 s (DPS, 1996) route optimisation software was used for the analysis of routes. The route choice between call points, as well as the visit order for call points, could be optimised with respect to time, distance or cost. In this study, optimisations were made with respect to time, which was assumed to imply that excessive use of narrow roads would be avoided in the proposed solutions. Time and distance were calculated for each registered route and compared to driving time duration. Twenty-two routes were recorded, all with start and stop at the abattoir. However two of the routes involved only one collection point each and were not included in route analysis. There was also one invalid GPS registration and it was unusable for route choice analysis. Therefore in total, 19 routes were analysed.

3.2 Vibration measurement on vehicles and animals

Vehicles and sensors

Cattle transporter vehicle (Volvo FM 12 4x2) and equipped with two pens separated by a steel gate was used for the field experiment. Dimension of the observation box was 2.45m x 2.50 m (front pen). Vibration measuring devices, Acc1 and Acc2 were mounted on the chassis and floor of the vehicle respectively and Acc3 on the animal. The sensors Acc1and Acc2 were connected to a computer by cable and measurements were made at a sampling frequency of 1000 Hz for three channels (vertical, horizontal and lateral directions), i.e. 333 Hz per channel. Rolab vibration sensor unit described earlier (Gebresenbet et al., 2005) was used to measure vibration levels on the animals. It was a tri-axial accelerometer. The sensor measures acceleration in a range of frequency from 0 to 500 Hz with a full-scale range of ±10g. Vehicle speed, geographical location of animal collection points (farms), delivery point (abattoir), slope and undulation of roads and route were recorded during each journey using an antenna placed on the cab roof and connected to a GPS (Magellan 315) signal receiver by cable. The information was then transferred to a computer in the cab by cable from the GPS unit.

Vibration sensors (Cargolog FAT 90V2) were also mounted on floors of cattle (Volvo FM 12 4x2) and multi-floor pigs transport vehicles. The vehicles have air suspension systems. The vibration sensors were mounted in observation box located near the cabin (cattle) and on floors one and three of pigs transport vehicle. The devise is a battery-powered designed to multifunction electronic logger system (Mobitron, 2009). The system consisted primarily of two parts: portable, battery-operated recording and communication units. A PC interface unit was used to communicate with the recording unit to initiate sampling sequences and download the collected data after recording periods.

Vibration measurement

Measurement of vibration was conducted on cattle transport vehicle and on dairy cows. In total 95 dairy cows, five per each trip were used in field experiments. The logger containing sensors was mounted with tape on a girth belt around each animal chest. Each sensor measures acceleration with a full-scale range of ±10 m s^{-2} and can measure both dynamic and static acceleration in the frequency interval 0-500 Hz. Each measurement was triggered manually via the cab computer and data could be transferred wirelessly between logger and computer by a radio signal system. The vibration equipment picked up signals from a transmitter in the stock crate via an antenna mounted centrally on the ceiling and measured automatically for 20-second periods with the same sampling frequency as the Acc1 and Acc2 sensors. During the field experiment, only some data were transferred to the computer. Most were saved in the logger itself and transferred to the computer at the end of each experiment.

The experimental design consisted of: 4 speeds x 4 road types x 2 standing orientations x 1 animal category (cows) x 2 replications.

During transport continuous recording of acceleration signals were conducted on cattle and pigs transport vehicles all along the journeys so as to adequately describe vibration on the vehicles as well as related to farm animals behaviours.

The second vibration experiment was carried out as a component of a comprehensive field experiment designed to study the effect of transport time on welfare of the animals and therefore has the same experimental design as transport time.

The experimental design: 3 transport time x 3 animal category x 2 seasons x 3 replications. Transport times were 4, 8 and 12 h including seasons winter and summer. Animal categories were cows, bulls and pigs.

Analysis

Vibration data were frequency weighted, and prior to data analysis, the raw data were filtered using a second order Butterworth filter with low and high frequency cut-off of 0.1 and 250 Hz, respectively. The parameters, root mean square *(r.m.s)*, crest factor (*CF*), vibration dose value (*VDV*), estimated vibration dose value (*eVDV*), transmissibility, 8 h vibration exposure and power spectrum density (*PSD*) were then evaluated. All analyses were performed using Matlab software (version R2009b). Statistical data analysis has also been conducted with SAS (version 9.3) software, using GLM and ANOVA procedures.

3.3 Effect of transport times on welfare of animals

Vehicles and sensors

Observation boxes of pigs and cattle transport vehicles were equipped with temperature and relative humidity sensors (Cargolog FAT 90V2) and video cameras to monitor behaviour of the animals during transport. Cattle transporter vehicle was a single deck and the observation box was close to the cabin. In pigs transport vehicle the observation pen was located on the third floor. When animals were in transit, provision of ventilation occurred through apertures positioned along the length of the vehicles body. The openings on each vehicle along the sides of the walls could be adjusted manually depending on the weather conditions and the area of the openings complies with Swedish Department of Agriculture (SJVFS 2000:133). It states that, as a rule of thumb the vent opening area should be about 20% of the vehicle floor area. The floors were covered with a rubber mat and sawdust was spread over the floor of approximately 20 mm thick before the start of loading. The trucks used for cattle and pigs transport were equipped with adjustable loading ramps. During loading and unloading events animals behaviour was recorded using a hand held camera (Sony DCR-HC47E) and visual observations.

Animals and parameters

In total 162 animals (82 cows and 80 bulls) were transported in the observation pen and used for the field experiment. The average weight of the animals was in the range of 450 to 550 kg. Blood samples were collected from the animals and behavioural studies were made on them. Blood samples were also taken for control purpose, from 20 animals, 10 bulls and 10 cows that were not in transit (from various farms). EU regulation regarding space requirements for medium size cattle (550 kg) travelling by road or rail is 1.3 – 1.6 m^2 $animal^{-1}$ (EU, 2005). Transport time was animal category based so cows and bulls were transported separately.
The number of transported pigs from various farms to the abattoir was 2753 in total. Out of these 216 pigs were transported in the observation box and behavioural study was made on them. Blood samples were collected from 90 pigs for field experiment, and blood samples were also collected for control purposes from 20 pigs that were not in transit. Last feeding period for the pigs to be transported was at least 4 h before transport. The mean space allowance was 0.55 m^2 pig^{-1}. The standard space allowance according to EU regulation of 2005 is 0.51 m^2 pig^{-1} weighing up to 100 kg. The age of the pigs was six months and their average weight was about 100 kg.

Blood parameters

Blood sample were taken at the farms before transport and at the abattoir immediate after stunning for determination concentration levels of cortisol, creatine kinase, glucose and lactate. Therefore every animal was bled twice, at the farm and at the slaughter house. In cows and bulls, blood samples were collected from the coccygeal vein, while in pigs jagular vein was used. Collected blood samples were centrifuged for at least 10 min at 2000 rpm at room temperature. Separated cells and plasma were removed using Pasteur pipettes, placed in 1.5 mL micro tubes and stored at -20 °C until analysis.

Meat pH value

Meat samples were taken from the longissimus dorsi (LD). The carcases were chilled for 24 h at +4°C. During that time, temperature and pH decrease were measured at 5, 12, 18 and 24 h post-mortem.

Behaviour

Cows, bulls and pigs behaviours were continuously observed and documented at farms (during blood sampling and loading), in transit and during unloading at the abattoir by visual observation, portable and fixed video cameras. To evaluate behavioural alterations in response to handling and transport activities, based on literature and experiences definitions were given to the most common observed behaviours. In the study duration of behavioural events were included in a more accurate approach to demonstrate the severity of each factor that caused behavioural alterations. To determine frequency, occurrence of behavioural events and total number of animals in the observation box were considered. Behaviours of the animals behaved in various ways, some of them could occur several times during a given transport time and when expressed as the ratio of behavioural occurrence to the total number of animals in the observation pen, it was considerably higher than the number of animals. But other behaviours occurred to a lesser degree and their ratio remained more or less close to the number of animals. Occurrences of behaviour and duration of events were determined from video recordings and documentations of observation made during the field work. The final quantified behaviour was expressed as the product of frequency of events and duration of events.

$$Frequency = \frac{A}{B} \qquad (1)$$

$$Behaviour = Frequency \times t \qquad (2)$$

Where:

A: Occurrence of behaviour;   B: Total number of pigs in the observation box
t: Duration of events in minutes

Temperature and relative humidity

Temperature and relative humidity were the considered parameters during transport of farm animals. During winter and summer transport, the animals were exposed to a wide range of ambient temperature and relative humidity variations. Temperature and relative humidity inside container of the vehicles were measured simultaneously and continuously throughout transport time. Thermal-humidity index (THI) was later calculated. THI is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. It was calculated to determine the level of thermal stress using the equation:

$$THI = 0.8\ t_{ab} + RH\ (t_{ab}-14.4) + 46.4 \qquad (3)$$

Where t_{ab} is dry bulb air temperature (^oC) and RH is relative humidity

Table 1. *Experimental design of transport time.*

Factors	Category	Description
Vehicle	1	Vehicles with ventilation
Transport time	3	4, 8 and 12 h
Animals	3	Cows, bulls and pigs
Seasons	2	Winter and summer

The experimental design included 5 animals (for each trip) x 3 transport times x 3 animal categories x 2 seasons and 3 replications.

Analysis

Statistical data analysis has been made separately for blood parameters, behavioural, pH and air quality with SAS 9.2 statistical package PC-based program. Multivariate analysis was also done together for blood parameters and behaviours using General Linear Model (GLM) and clustering (dendrogram).

4 Results

4.1 Logistics chain of animal transport and abattoir operations

Description of operation

Seasonal and short-term variations in supply and demand, created challenges to the planning of cattle transport operations. Cattle were delivered to slaughter for three reasons; slaughter weight reached for meat production, milking cows removed from production due to lameness and other diseases, or the entire production at a farm closed down.

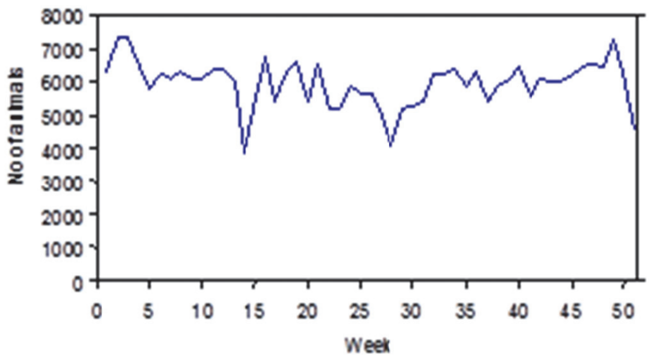


Figure 3. No. of animals delivered weekly to Swedish Meats, Uppsala, during one year.

The planning of deliveries under unpredictable conditions (inherent from especially the latter two of the above mentioned reasons) required some flexibility in animal transport and abattoir. The seasonal variations in cattle transport to Swedish Meats abattoir in Uppsala is indicated in Figure 3. Transport of animals to the abattoir located in Uppsala was operated by contracted transport operators. The geographic area supplying animals to the abattoir was divided into sectors, with one transport operator assigned to each sector. The long term planning of operations was based on a pre-registration of delivery information received from producers. However the detailed transport planning and scheduling was performed by the transport operators for their respective geographic areas (Figure 4).

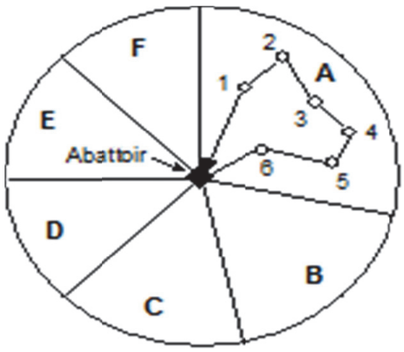


Figure 4. Principle for organisation of animal transport into sectors (A-F), each assigned to one transport operator; nodes (1-6) indicate farms visited on a fictive collection route in sector A.

Vehicle activity chain

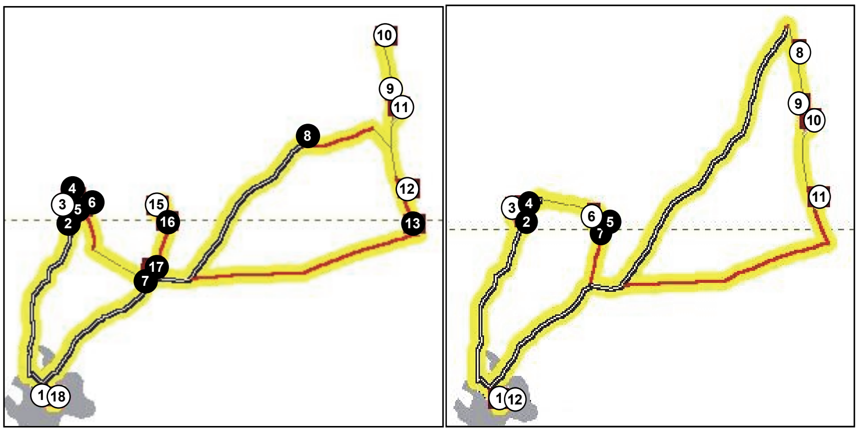
According to observations of 22 routes involving in total 90 collection stops at farms, the effective average loading time was 12.7 minutes, while the time required for preparations before and after loading (i.e. parking and preparing the vehicle and contacting the farmer), was 6.5 minutes. Observations of unloading included 61 deliveries and the duration of unloading, including waiting time and preparation, varied between 7 and 98 min, with an average of 23.7 minutes. The effective unloading time (waiting time and preparation excluded) was 16.7 minutes. Waiting and preparations before unloading took on average 6.3 min, but delays of up to 85 min were observed. Queues were said to occur at around 20%, or less, of the deliveries at the abattoir, and likewise before vehicle wash. Waiting times in case of queues were estimated by the drivers to be on average 24 min (max 80 min) before unloading and 30 min (max 80 min) before washing. Important factors behind the build-up of queues were vehicles arrivals before or after the assigned or expected time, and the stable capacity was limited to 350 pigs. After unloading, washing the vehicle took 60 min (varied between 30 and 150).

Animal activity chain

After unloading, cattle were moved either directly to the stunning point, or to lairage boxes. The reasons for keeping cattle in lairage were that the slaughter capacity was temporarily filled, or the cattle arrived after slaughter chain was closed for the day. In the lairage box for 55 observed cases, animals waiting time was on average 43 minutes. For 15 of the same animals (27%), lairage time exceeded 1 h (maximum was 2.2 h). During a technical breakdown in the slaughter chain, for 17 observed animals the lairage times increased to on average of 3.8 h; minimum 2.2 h and maximum 6.5 h.

Route optimisation

The differences between registered and calculated driving times for the 19 routes were approximately normal distributed around an average of 6.4% of recorded driving time. Although for most routes the optimised route was identical with the drivers' route choice, but the analysis revealed potential savings of certain routes of up to 23%. One of the routes with 15% potential savings is displayed in Figure 5.



(a) (b)

Figure 5. (a) registered route no.14 (3 h 14 min driving time, 174 km distance), (b) optimised route (2 h 45 min, 153 km); 1, call points (departure, collections and delivery); 1, reference points.

4.2 Vibration levels and frequencies on vehicles and animals during transport

Effect of speed on vibration

A plot of vertical chassis acceleration against a range of speeds between 14 and 92 km h^{-1} produced an exponential equation with $R^2 = 0.92$ (Figure 6), where the experiment was done on an asphalted road. Figure 6 depicts a typical speed and vibration profile. Prior to the truck starting moving, the vibration level was about 0.5 m s^{-2} due to animal movement and engine running. Once the truck started moving, the vibration levels followed the speed curve profile.

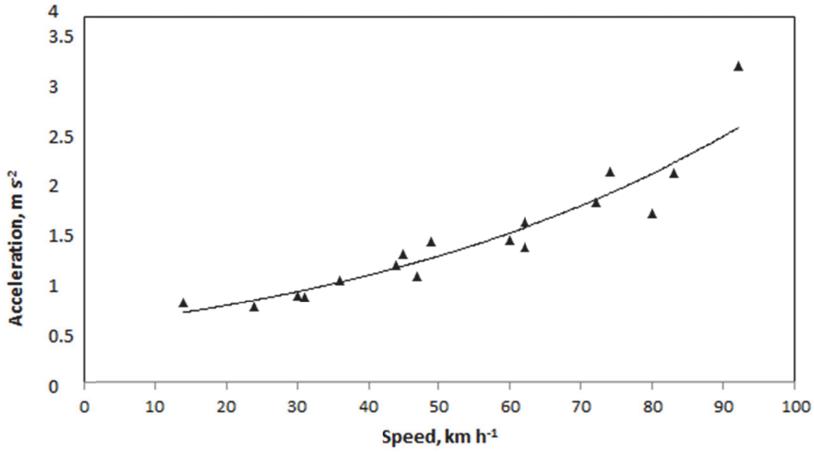


Figure 6. Vertical vibration on a chassi as a function of vehicle speed and described well with the equation, $a = 0.6035e^{0.0167v}$ and correlation of determination $R^2 = 0.92$

Effect of speed, road and standing orientation on vibration

Acceleration data for different road conditions are presented in Table 2. Data for animals facing forward (FF) are lacking for speeds of 30 and 50 km h^{-1} on roads RT1 and RT1g. Data for the speed of 90 km h^{-1} on gravel road is also missing, as it was not allowable according to traffic regulation. Animals experienced the highest levels of vibration on gravel roads at 50 km h^{-1} (2.23±0.27 m s^{-2}) and 70 km h^{-1} (2.27 ± 0.33 m s^{-2}) in the driving direction, when the animals were positioned perpendicular to the direction of movement (PP). The levels of vibration (0.81 ± 0.5 and 0.8 ±0.45 m s^{-2}) were also highest for the lateral axis at these speeds and road conditions. At 70 km h^{-1} and rough road surface (RT1g), the vibration level was 2.27 ±0.33ms^{-2}, but at 90 km h^{-1} and good road quality (RT3) it was only 1.6 ±0.24 m s^{-2} (Table 2). Considering standing orientation, animals facing in the driving direction (FF) were generally exposed to higher horizontal vibration levels than animals facing perpendicular (PP) to the driving direction, for all roads and speeds. The same was true for lateral vibration levels, but not for vertical vibrations. Standing orientation, for all roads and speeds, animals facing in the driving direction (FF) were generally exposed to higher horizontal vibration levels than animals facing perpendicular (PP) to the driving direction (Table 2).

Table 2. *Mean acceleration levels, with standard deviation, on animals at different speeds, road types, standing forward (FF) and perpendicular (PP) to the driving direction.*

Speed Km h^{-1}	Road type	Standing orientation	Vertical m s^{-2}	Horizontal m s^{-2}	Lateral m s^{-2}
30	*RT1*	PP	0.56±0.01	0.85±0.12	0.38±0.04
		FF	-	-	-
50	*RT1g*	PP	0.3±0.28	2.23±0.27	0.81±0.5
		FF	-	-	-
	RT1	PP	0.52±0.26	1.08±0.69	0.26±0.15
		FF	-	-	-
	RT2	PP	0.48±0.21	1.09±0.31	0.42±0.66
		FF	0.49±0.5	1.54±0.45	0.55±0.42
70	*RT1g*	PP	0.35±0.92	2.27±0.33	0.8±0.45
		FF	-	-	-
	RT1	PP	0.31±0.29	0.87±0.09	0.35±0.23
		FF	-	-	-
	RT2	PP	0.49±0.58	1.08±0.55	0.38±0.16
		FF	0.47±0.42	1.51±0.5	0.5±0.3
	RT3	PP	0.34±0.44	1.1±0.36	0.48±0.25
		FF	0.33±0.4	1.54±0.49	0.53±0.45
90	*RT2*	PP	0.36±0.24	1.6±0.24	0.54±0.1
		FF	0.36±0.34	1.45±0.58	0.58±0.58
	RT3	PP	0.32±0.19	1.16±0.32	0.51±0.26
		FF	0.30±0.4	1.52±0.53	0.54±0.46

Transmissibility

To study the effectiveness of vibration isolation, transmissibility was estimated using acceleration, VDV and r.m.s between the chassis (input) and the floor (output), and the ratio of floor (input) to cattle (output) was calculated. The values were expressed as ratios of output to input in their respective locations and axes. Transmitted acceleration, VDV and r.m.s from chassis to crate's floor were 55-73%, 39-56% and 48-66%, respectively. However, transfer of vibration from floor to animal was 100-158% for acceleration, 101-154% for VDV and 95-143% for r.m.s (Table 3).

Vibration exposure for eight h transport

Frequency weighted r.m.s and exposure values over an 8 h transport period were calculated and differed for floor and animals, with the values in the horizontal (0.92 m s^{-2}) and lateral (1 m s^{-2}) directions being higher on animals than on the floor (Table 4).

Table 3. *Transmissibility of acceleration, VDV and r.m.s from chassi to floor and floor to cattle.*

	Floor			Cattle		
	Chassi			Chassi		
	Vertical	Horizontal	Lateral	Vertical	Horizontal	Lateral
a	0.55±0.15	0.59±0.13	0.73±0.1	1±0.1	1.3±0.4	1.58±0.36
VDV	0.39±0.14	0.42±0.12	0.56±0.11	1.01±0.36	1.14±0.39	1.54±0.54
r.m.s	0.48±0.15	0.51±0.13	0.66±0.12	0.95±0.18	1.1±0.37	1.43±0.32

Table 4. *Cattle and floor vibration exposure during 8 hours transport time (Tarmac road, TR2).*

	Vertical		Horizontal		Lateral	
	r.m.s ms^{-2}	Exposure ms^{-2}	r .m.s ms^{-2}	Exposure ms^{-2}	r.m.s ms^{-2}	Exposure ms^{-2}
Animal	0.63±0.13	0.61±0.12	0.68±0.25	0.92±0.35	0.75±0.16	1±0.21
Floor	0.67±0.03	0.64±0.03	0.63±0.03	0.85±0.04	0.51±0.03	0.7±0.04

4.3 Vibration on animal transport vehicles and related animals behaviour

Pigs transport vehicle

To evaluate vibration transmissibility of pigs transport vehicle, acceleration, r.m.s and VDV were expressed as the ratio of input (floor 1) to output (floor 3).

Table 5. *Transmissibility of acceleration, r.m.s and VDV from floor 1 to floor 3.*

	Vertical	Horizontal	Lateral
a, ms^{-2}	1.1 ± 0.02	0.88 ± 0.08	1.13 ± 0.07
r.m.s , ms^{-2}	1.1 ± 0.02	0.87 ± 0.03	1.11 ± 0.05
VDV, $ms^{-1.75}$	1.08 ± 0.13	0.84 ± 0.11	1.12 ± 0.16

Ratio of transmitted acceleration was in the range of 110% to 113% in vertical and lateral orientations and in horizontal it was 88%. In vertical and lateral axes transmissibility estimated using r.m.s varied between 110% and 111%, respectively, however in the horizontal axis, the value was only 87%. Ratio of VDV was 84% in horizontal axis and in vertical and lateral directions 108% and 112% respectively (Table 5).

Effect of vibration on pigs behaviour

Transported pigs displayed travel sickness occurred as retching and vomiting during short (4 h), medium (8 h) and long (12 h) transport time (Figure 7). Against transport time, retching and vomiting behaviour could be described in a linear form. These behaviours were often coupled with other symptoms of travel sickness like chomping and foaming at the mouth.

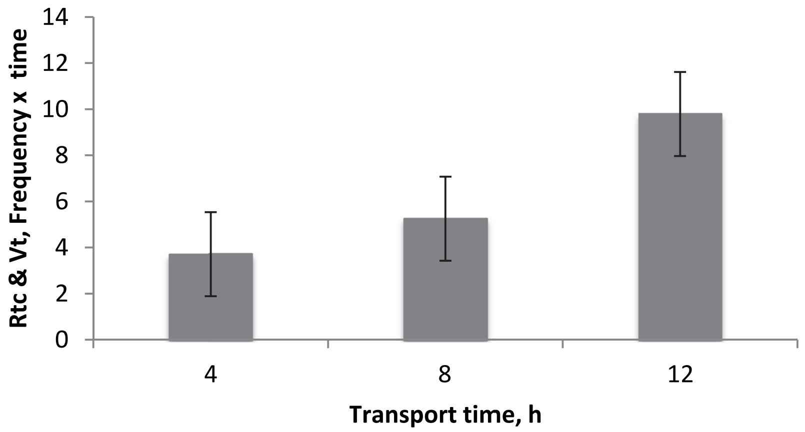


Figure 7. Mean and standard deviations of retching (Rtc) and vomiting (Vt) of pigs during transport.

During 4, 8 and 12 h transport time over 85% (P < 0.006) of the pigs in the observation box preferred to lie in the driving direction. About 10% of them lied perpendicular to the driving orientation and supported themselves on the side walls of the container. In this case, lying included fully lying on the floor or on other animal or partly supported by fore legs. Lying in the horizontal orientation was characterized by very close body contact of the animals and they swayed in group due to the effect of vibration.

Driving performance

To study the effect of driving skill on vibration level comparison was made on short trips and two farms were considered. The distance between the farms was around 600 m. Therefore, routes from both farms were almost the same. Two trips for each driver were conducted from each farm to an abattoir located in Southern Sweden. Duration of the transport was the same for all trips (4 h), in spite of that vibration exposure could differ from one other (Figure 8).

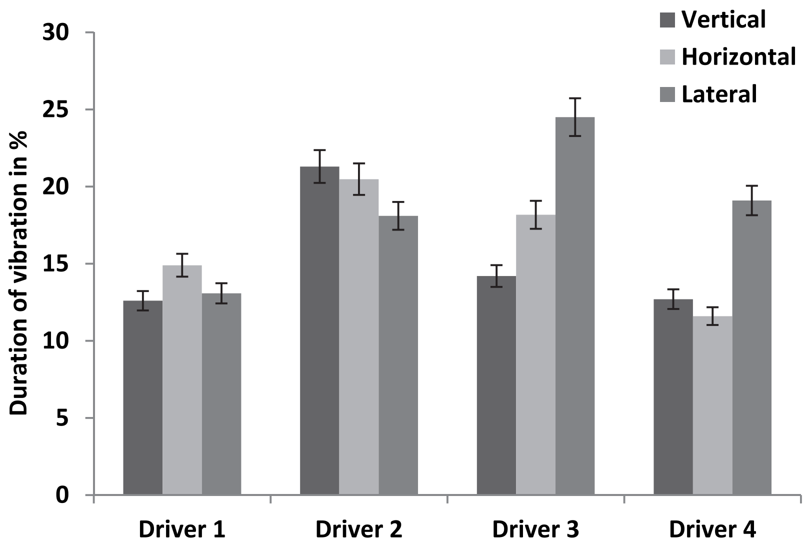


Figure 8. Mean and standard deviations of drivers'performance and occurrence of vibration on floors 1 and 3 of pigs transport vehicle.

Vibration related cattle behaviour

During transport cows and bulls exhibited certain behaviours associated with vibration. Swaying and loss of balance were the most common vibration related behaviours and are illustrated in Figure 9 and 10. These behaviours could constantly increase in both bulls and cows in different proportions. Bulls' swaying behaviour increased in an exponential form but for cows, the growth was in a linear form against transport time. Loss of balance increased with transport time in a linear for bulls and in an exponential form for cows.

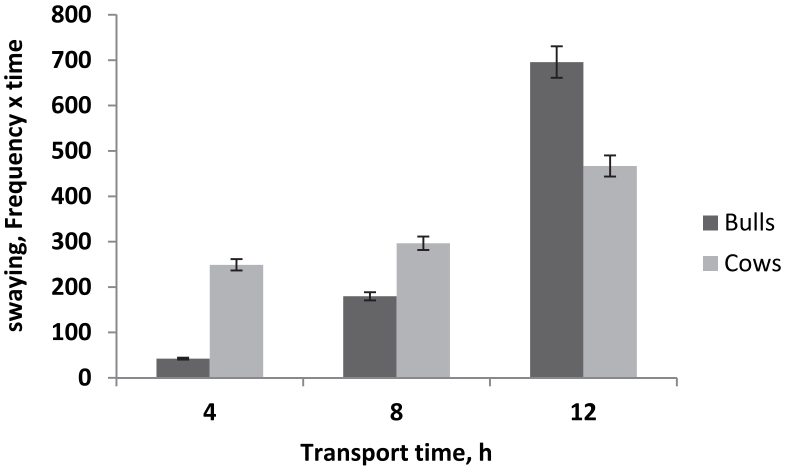


Figure 9. Mean and standard deviations of swaying of bulls and cows at various transport time.

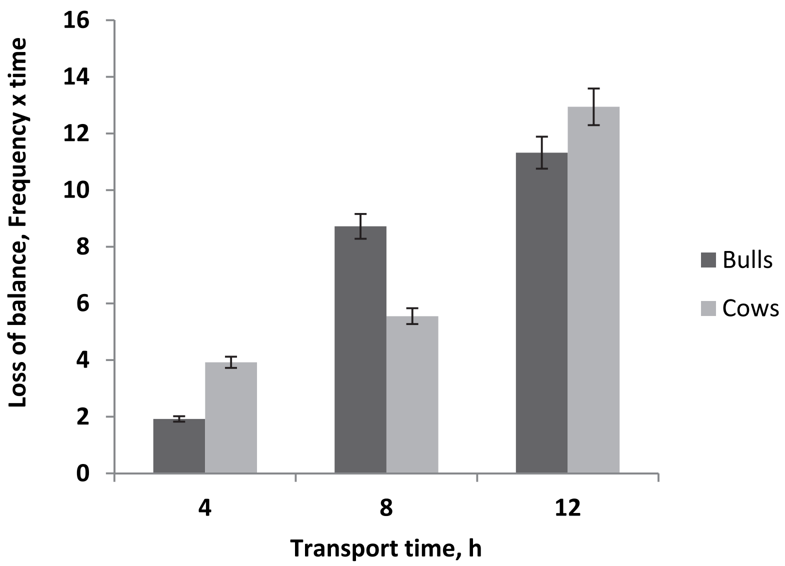


Figure 10. Mean and standard deviations of loss of balance of bulls and cows at various transport time.

4.4 Effect of transport time on welfare of pigs

Loading and unloading times

Average loading and unloading times during winter were 0.65 and 0.16 min pig^{-1} respectively. However, during summer loading and unloading times were 0.35 and 0.12 min pig^{-1} respectively.

pH measurement

A summary of the mean pH values are presented in Table 6. The highest pH_{24} values for summer and winter were 5.99 ± 0.29 and 5.58 ± 0.14, respectively. Seasonal effect on pH values are indicated in Figure 11 at pH_0, pH_5, pH_{18} and pH_{24} post mortem h. During winter measurements, the values of pH_0 and ultimate pH ranged from 6.5 to 5.5, whereas summer pH values varied between 6.29 and 5.99.

Table 6. *Summary of pH values for different seasons and transport time.*

Season	Transport time (h)	pH_0	pH_5	pH_{18}	pH_{24}
	4	6.54 ± 0.32	6.15 ± 0.25	5.67 ± 0.21	5.65 ± 0.17
Summer	8	6.42 ± 0.31	6.23 ± 0.23	5.69 ± 0.23	5.79 ± 0.18
	12	6.29 ± 0.28	6.10 ± 0.19	5.59 ± 0.19	5.99 ± 0.29
	4	6.50 ± 0.08	6.49 ± 0.13	5.46 ± 0.12	5.58 ± 0.14
Winter	8	6.50 ± 0.17	6.37 ± 0.22	5.41 ± 0.21	5.48 ± 0.12
	12	6.50 ± 0.21	6.23 ± 0.23	5.53 ± 0.19	5.50 ± 0.22

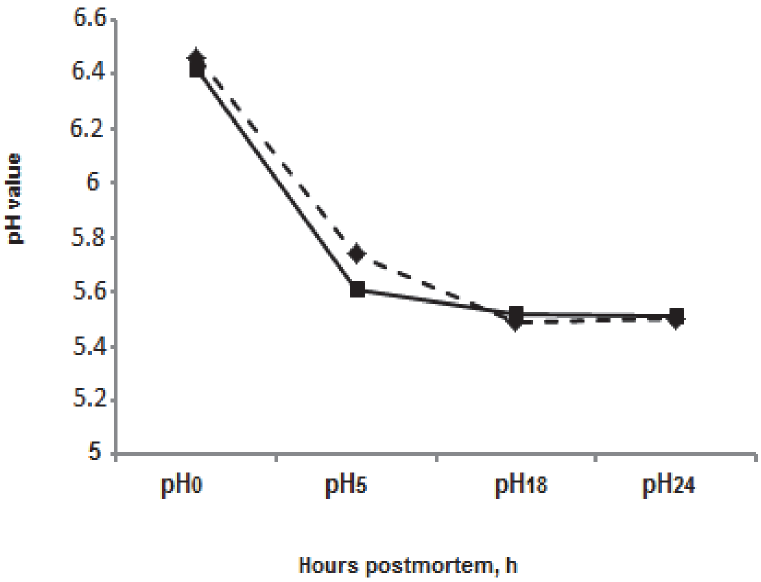


Figure 11. pH values in pig carcases during winter and summer after 12 h transport. Dashed and continuous lines denote summer and winter respectively.

Blood parameters

Time of collecting blood samples from control and transport animals was the same. The main purpose of collecting blood samples from control animals was to use the blood hormones as reference for the values of transported pigs. Concentration level of cortisol ranged from 105 to 165 nmol L^{-1} lowest at (night time) 23:00 h and highest at (day time) 6:00 h respectively. Glucose and lactate concentration levels for control animals were 3.5 - 6.6 mmol L^{-1} and 4.2 - 7.3 mmol L^{-1} respectively. Control values of creatine kinase ranged from 115 to 129 µmol L^{-1}.

Elevation of cortisol concentration during short (4 h) transport was highest and decreased with an increase of transport time. The rate of elevation (58.2-25.3 nmol L^{-1}, winter; 59.2-31.8 nmol L^{-1}, summer) was generally inversely proportional to transport time for the range of transport time used for this study ($P<0.001$).

The effect of transport time on concentration level of blood glucose was significant ($P<0.01$). Glucose concentration increased from short to medium duration and decreased thereafter. Therefore it was highest at 8 and lowest at 12 h transport time for winter and summer experiments (Figure 12). During 8 h transport time, the maximum concentration noted was 20.46 mmol L^{-1} and that was 3 fold more than the reference value.

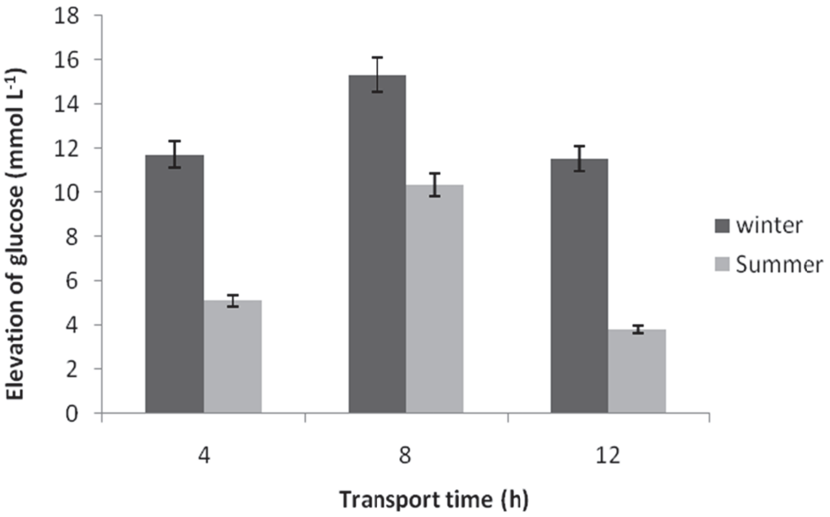


Figure 12. Mean and standard deviation of glucose concentration level during winter and summer for different transport times.

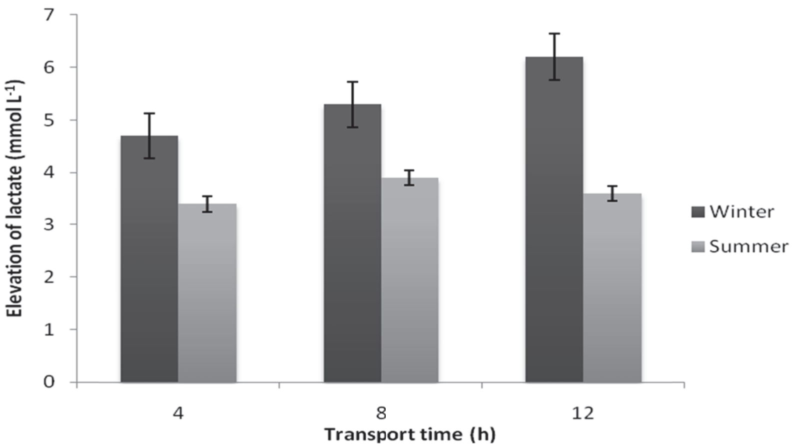


Figure 13. Mean and standard deviation of lactate concentration during winter and summer for 4, 8 and 12 h transport time.

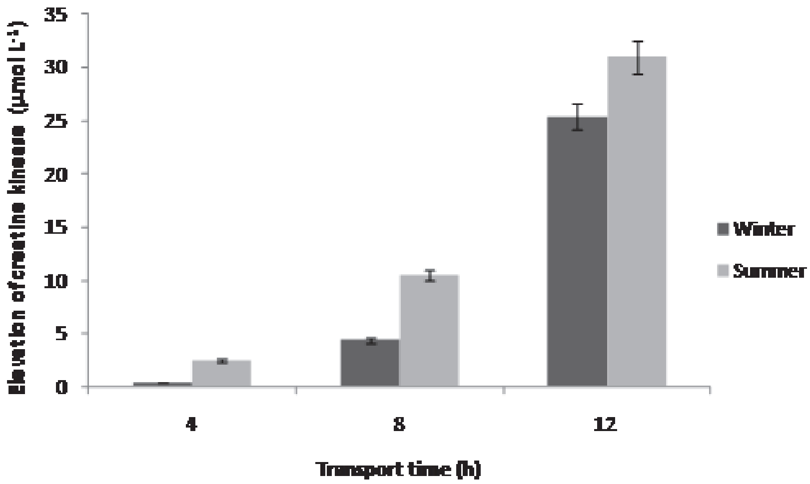


Figure 14. Mean and standard deviation of creatine kinase concentration level for winter and summer increased exponentially with transport time.

Concentration levels of lactate (winter) in the blood increased (4.7 - 6.2 mmol L^{-1}) with an increase in transport time and (Figure 13) and positively correlated ($P < 0.002$). Concentration level in summer was highest at 8 h (3.9 mmol L^{-1}) and lowest at 4 h (3.4 mmol L^{-1}) transport time, unlike winter measurement the variations were small.

The elevation in concentration levels of creatine kinase ranged from 0.4 to 25.4 (winter) and 2.5 - 31 (summer) µmol L^{-1} and positively correlated with transport time ($P<0.002$). The relationship between concentration level and transport time was exponential ($R^2 = 0.99$) for both seasons. The rate of increase from 4 to 8 h was lower than from 8 to 12 h transport time (Figure 14). During summer at 12 h transport time, the maximum value after transport was 154 µmol L^{-1} and exceeded the reference value of 129 µmol L^{-1}.

Behavioural observation and quantification

Lying, sitting, rooting, vocalization, restless and change of position, smelling, panting, loss of balance and fighting were significant and positively correlated with transport time ($P < 0.009$). About 50% of pigs were lying about two h after the vehicle started moving. About 80% of pigs were lying after 6 h transport time, and the behaviour remained similar until 12 h (Table 7 and Figure 15).

Table 7. *Observed behaviours during 4, 8 and 12 h transport times.*

Behaviour	Transport time (h)					
	4		8		12	
	Freq	Freq × Time	Freq	Freq × Time	Freq	Freq × Time
Ft	0.62	0.16	1.37	0.21	1.62	0.23
Jn	0.05	0.05	0.22	0.22	0.43	0.32
Ln	0.07	0.31	0.14	0.46	0.19	1.25
Ls	0.48	0.21	0.71	0.43	0.86	0.94
Ly	2.71	62.63	5.93	812.09	8.33	1,269.25
Pt	0.01	0.02	0.10	0.55	0.40	0.86
Rc	0.85	2.29	0.95	3.62	1.67	3.67
Rt	1.26	3.68	1.43	4.72	2.52	15.67
Sm	0.74	2.33	1	4.17	1.33	4.299
St	0.95	4.19	1.43	7.18	1.59	15.09
Vc	3.1	3.72	4.8	5.3	7.6	8.15

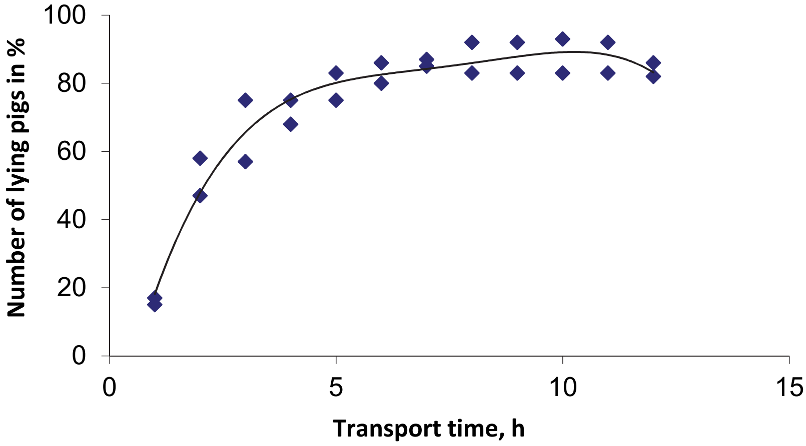


Figure 15. Proportion of pigs lying depending on transport time, described with 4th order polynomial function, with coefficient of determination, R^2= 0.95.

Temperature, relative humidity and THI

Number of pigs in the vehicle was 190, lower than the average (192) and loading time was 0.38 min pig^{-1}, greater than summer average loading time (0.35 min pig^{-1}). Figure 16 depicts where both temperature and relative humidity were high before loading started. At the beginning, temperature was 21.5 ^{0}C and continued rising to 23 ^{0}C until loading was completed. Roughly after 1 h trip temperature decreased to 11 ^{0}C and thereafter the range was from 11 to 20 ^{0}C until unloading time.

Relative humidity was 85.2% at the beginning of loading and reached the highest level at loading time. After loading it decreased sharply for a short while and again went up to the maximum level and it could decrease after about 6 h transport time.

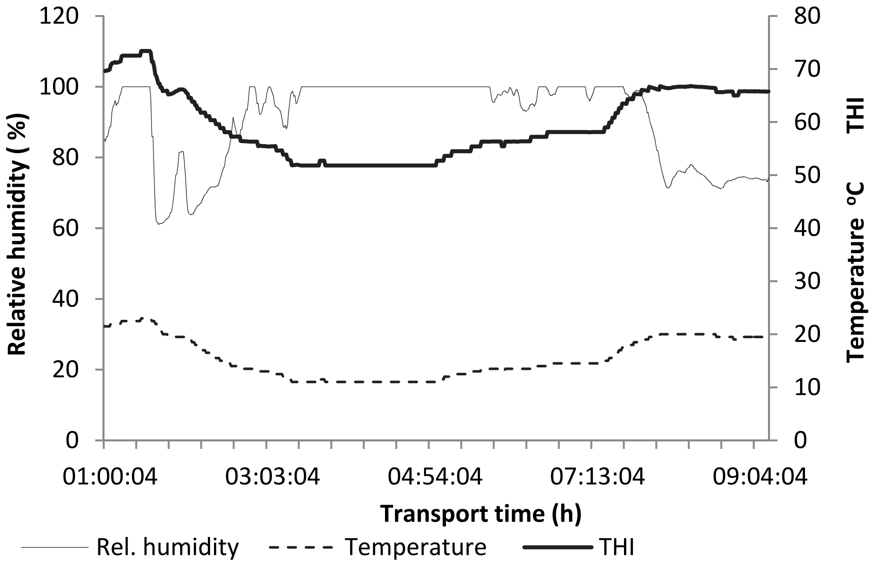


Figure 16. Temperature, relative humidity and THI variations during 8 h transport time (summer).

Figure 17 illustrates the case of low relative humidity and high temperature at the initial case and 198 pigs were loaded in the vehicle and loading time was 0.4 min pig^{-1}. Temperature was 20.5 ^{0}C during loading and didn't show further increase. During transport it could decrease to 16 ^{0}C and for the whole trip the range was 16 to 21^{0}C.

Initial relative humidity of the vehicle at loading time was 58.4%, and the increase of humidity was not rapid and sharp; therefore it reached the maximum level after 70 minutes. After about 3½ h trip, the level of relative humidity started to go down.

During field measurements the lowest temperature (-2 ^{o}C) was recorded in February and the highest (28 ^{o}C) in July and the highest value occurred during loading event. In all measurements, whether temperature was rising or falling, relative humidity constantly increased during transport. In winter and summer experiments relative humidity reached its maximum value during each transport time. When initial relative humidity levels was below 60%, during loading, there was comparatively slow relative humidity growth and thereafter it decreased rather faster, as the vehicle started moving (compared to higher initial humidity).

Thermo-humidity index (THI) mean values of both seasons and for the three transport times (4, 8 and 12 h) were below the threshold value of 74 and the maximum mean value was 65.92. To study the effect of transport time on heat stress, values of THI within 4 h intervals (0-4, 4-8 and 8-12 h) of 4, 8 and12 h transport time were considered. The maximum, minimum, mean and standard deviations of values of each interval are reported in Table 8. Based on the recommended thresholds (Mader et al., 2006) THI values were grouped according to their level: normal (≤ 74); alert (75-78); danger (79-83) and emergency (≥ 84). The result showed that, during summer season the THI exceeded the normal value of 74. The maximum value observed was 79.2, which is in the danger zone (Table 8) and the incidence was during loading.

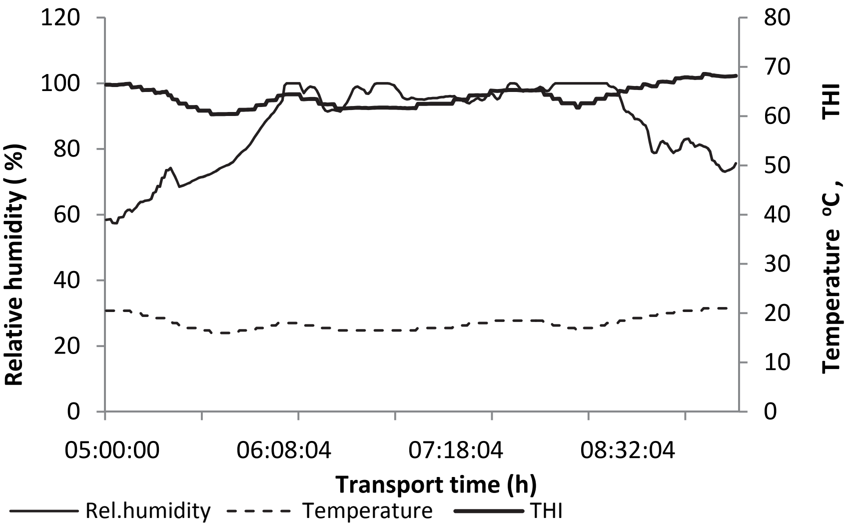


Figure 17. Temperature, relative humidity and THI variations during 4 h transport (summer).

Table 8. *THI values within the 0-4, 4-8, and 8-12 h intervals for all transport time in summer and winter.*

Season	Transport time (h)								THI
		0-4 (h)			4-8 (h)			8-12 (h)	
		Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Winter	4	50.2	37.5	41.1 ± 3.3						
	8	48.2	33.8	40.1 ± 3.2	41.5	38.3	39.2 ± 0.9			
	12	52	39.1	44.7 ± 3.4	43.9	37.8	41.2 ± 1.5	48	37.7	41.1 ± 1.6
Summer	4	70.4	58.9	62.4 ± 3.8						
	8	70.1	53.98	59.9 ± 5.5	22.7	54.5	59.3 ± 3.4			
	12	79.2	66.6	63.1 ± 4.9	63.9	54.5	59.8 ± 2.7	69.2	56.3	62.05 ± 4

Multivariate analysis

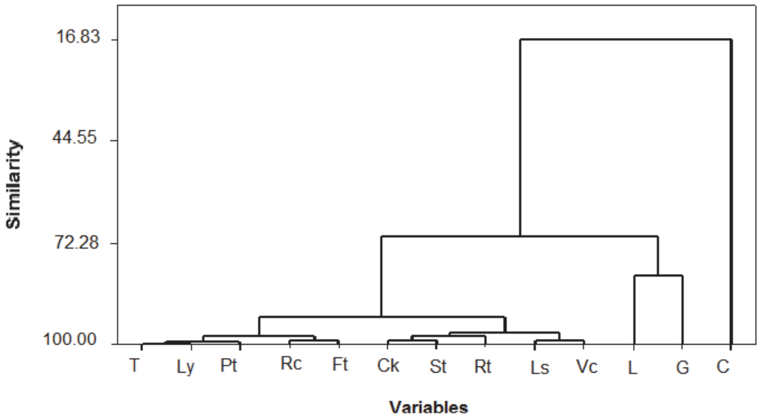


Figure 18. Dendrogram of transport time and stress related behaviour and blood hormones of pigs.

Figure 18 depicts dendrogram of transport time (T), blood parameters cortisol (C), glucose (G), lactate (L) and creatine kinease (Ck) together with behaviours. The included behaviours are lying (Ly), panting (Pt), restlessness and change of position (Rc), fighting (Ft), sitting (St), rooting (Rt), loss of balance (Ls) and vocalisation (Vc). Pair wise clusters were formed mostly among behaviours with the exception of transport - lying (T-Ly) and creatine kinease - sitting (Ck-St). Blood hormones lactate (L) and glucose (G) also formed a pair wise cluster. Bahaviours and enzyme creatine kinease formed the pairwise cluster at different levels but within the range of about 95 - 100% level of similarity. This indicates that the behaviours and creatine kinease were strongly correlated with transport. Lactate – glucose paired at 85% level of similarity and then sequentially added to the main clusters in addition to they are located apart from the origin indicating that their correlation with transport time was fairly strong. When cortisol (C) is sequentially added to the main clusters, it occurred at a wider level (16. 83%), demonstrating weak level of similarity. Behaviours and blood parameters (without cortisol) even though they formed pairs and similarities at various levels they indicated that transport was stressful to the animals.

4.5 Effect of transport times of up to 12 Hours on welfare of cows and bulls

Loading, unloading methods and time

Animal category, loading and unloading methods and time measured in minutes are illustrated in Table 9. On the farms, angle of loading ramp varied between 10^0 and 15^0 and during unloading the angle was 8^0 to 10^0. Loading to the vehicle and unloading times varied significantly ($P<0.002$) depending on keeping method (loose or tied) and animal categories. The highest and lowest loading time per animal was obtained for tied and loose bulls (6.5±3.7 and 0.77±0.49 min) respectively. In the case of cows loading time for tied was (3.43±1.74min) higher than for loose (2.73±1.57 min).

Table 9. *Loading and unloading time per animal and their ratios*

Activity	Cows		Bulls	
	Loose	Tied	Loose	Tied
Loading time per animal (min)	2.73±1.57	3.43±1.74	0.77±0.49	6.5±3.7
Unloading time per animal (min)	1.61±0.45	1.16±0.58	0.58±2.92	0.86±0.65
Ratio of loading/unloading	1.69±3.48	2.96±3	1.33±0.17	7.56±5.69

pH measurement

A summary of the mean and standard deviations of pH values are presented in Table 10. The final pH_{24} values for both winter and summer ranged from 5.58 to 5.28 in cows and 5.60 to 5.34 in bulls. Transport time has no any significant effect on pH_{24} values in any animal category.

Table 10. *Summary of pH values and their standard deviation for different animal categories, seasons and transport times, measurement at 0, 5, 18 and 24 h post mortem.*

Season	Animal	Transport time, h	pH_0	pH_5	pH_{18}	pH_{24}
Winter	Bulls	4	6.45±0.12	5.84±0.22	5.43±0.18	5.35±0.09
		8	6.61±0.11	5.90±0.25	5.78±0.14	5.56±0.11
		12	6.52±0.11	5.98±0.08	5.96±0.14	5.60±0.19
	Cows	4	6.49±0.23	5.76±0.16	5.53±0.11	5.28±0.22
		8	6.74±0.33	6.42±0.19	6.01±0.24	5.56±0.18
		12	6.12±0.20	5.56±0.22	5.36±0.22	5.35±0.16
Summer	Bulls	4	6.31±0.31	5.90±0.21	5.35±0.18	5.34±0.15
		8	6.74±0.33	6.42±0.19	6.01±0.24	5.56±0.18
		12	6.12±0.20	5.56±0.22	5.36±0.22	5.35±0.16
	Cows	4	6.18±0.21	6.46±0.23	5.59±0.15	5.58±0.11
		8	6.21±0.22	5.72±0.19	5.33±0.17	5.32±0.13
		12	6.39±0.18	5.74±0.17	5.44±0.16	5.40±0.14

Winter and summer 12 h cows transport time and pH values are presented in Figure 19. The initial pH values for winter and summer were 6.47 and 6.42 respectively and pH_5 value for winter (5.74) was higher than summer (5.61). The ultimate pH value for winter and summer was 5.5.

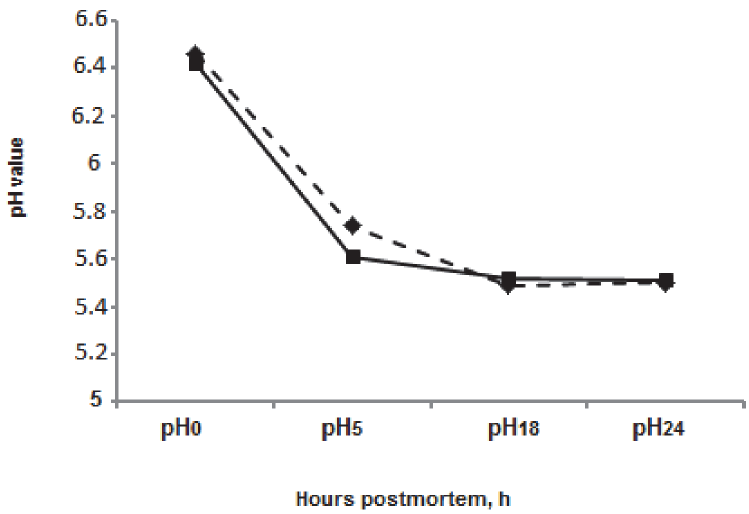


Figure 19. Typical pH values in cow carcases during summer and winter at 12 h transport time.

Control samples

The concentration levels of cortisol, glucose and lactate in control cows were 15 - 29 nmol L^{-1}, 5.4 mmol L^{-1} and 1.2 mmol L^{-1} respectively whereas creatine kinease concentration was 122 µmol L^{-1}. In control bulls the concentration levels of cortisol, glucose and lactate were 20 - 29 nmol L^{-1}, 4.7 mmol L^{-1} and 3 mmol L^{-1} respectively and the value for creatine kinease was 87.5 µmol L^{-1}. The values of cortisol were higher during the day and lower at night time for both animal categories.

Cortisol

In both bulls and cows during both seasons, the concentration of cortisol was significantly elevated ($P<0.001$) during short transport and decreased with an increase in transport time. During winter cortisol concentration levels in cows was highest at 4 h (118 nmol L^{-1}) thereafter it decreased to its lowest level at 8 h (43 nmol L^{-1}) and slightly increased at 12 h (48 nmol L^{-1}). Cortisol concentration for summer experiment was in the range of 80 to 14 nmol L^{-1} where the highest was at 4 and lowest at 12 h transport time. Summer concentration levels were lower at all transport time as compared with winter measurements as illustrated in Figure 20. Bulls cortisol concentration in winter behaved almost same as in cows. However during 12 h summer it could not decrease with transport time.

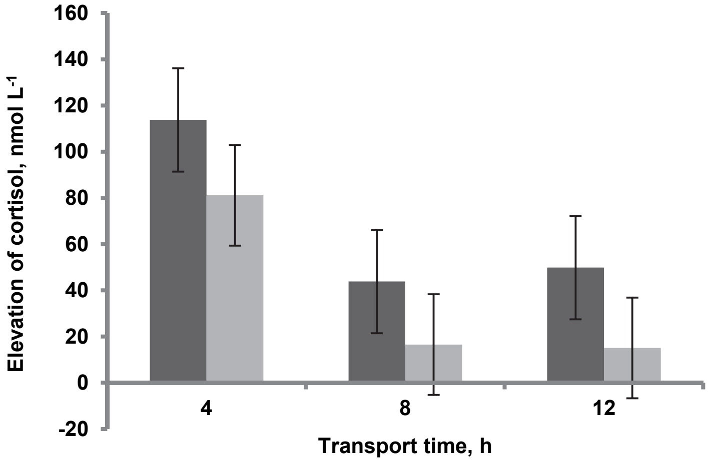
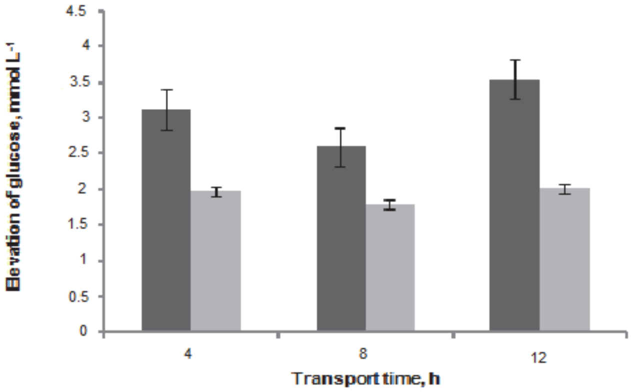


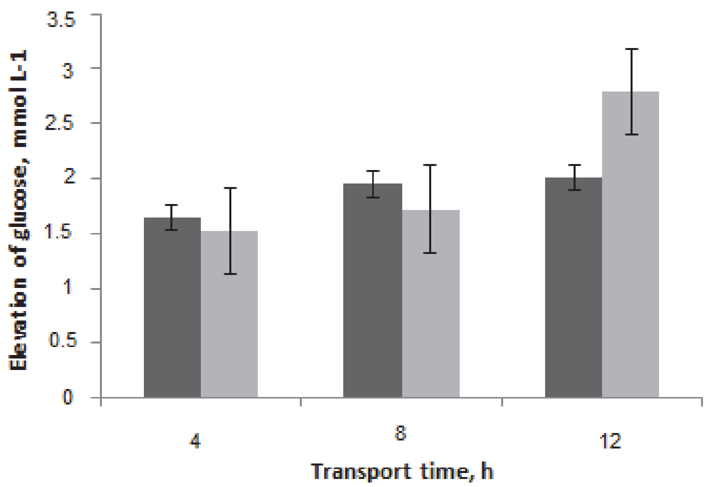
Figure 20. Means and standard error of cortisol concentration in cows for the different transport times and seasons. Dark and grey colours represent winter and summer respectively.

Glucose

During winter for cows concentration change in glucose was in the range 2.6 to 3.5 mmol L^{-1}. Highest level was at 12 and lowest at 8 h transport time and in summer the changes in concentrations at 4, 8 and 12 h transport time were 1.9, 1.8 and 2 mmol L^{-1} respectively. Even though the lowest and highest was at 8 and 12 h transport time the variation was marginal and generally the elevation levels were almost the same (Figure 21a). The effect of transport time on glucose concentration was significant ($P<0.01$) but didn't correlate with the increase in transport time. In bulls, changes in glucose concentrations were 1.6, 1.9 and 2 mmol L^{-1} at 4, 8 and 12 h transport time (winter) respectively. Thus there was only slight increase from 8 to 12 h transport time (Figure 21b). During summer glucose contentions were 1.5, 1.7 and 2.8 mmol L^{-1} at 4, 8 and 12 h transport time therefore lowest at 4 and highest at 12 h and increased with an increase in transport time ($p<0.02$).



(a)



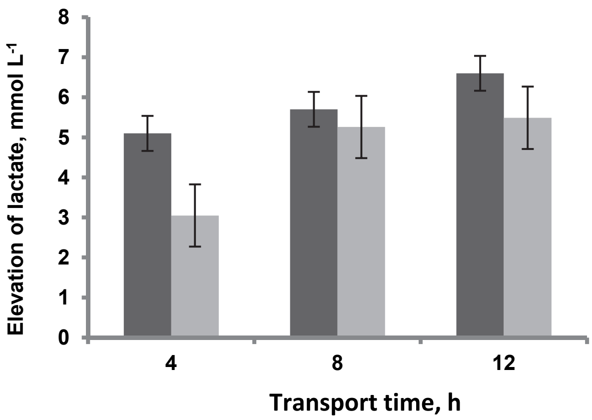
(b)

Figure 21. Mean and standard error of glucose concentration in cows (a) and bulls (b) for the different transport times and seasons. Dark and grey colours represent winter and summer respectively.

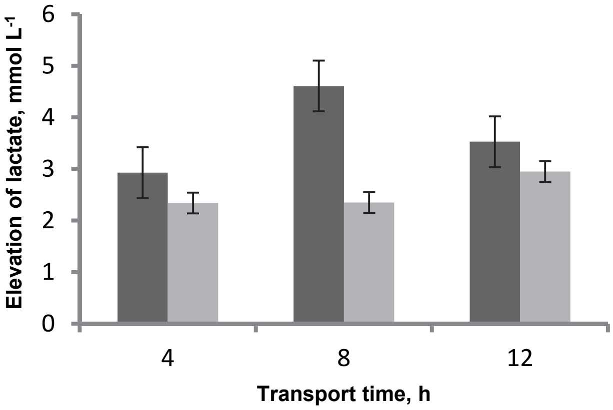
Lactate

In cows change in lactate concentrations were 5.7, 6.4 and 7.4 mmol L^{-1} for 4, 8 and 12 h transport time respectively (winter). Even during summer experiment lactate concentration were 3.6, 5.9 and 6.2 mmol L^{-1} at 4, 8 and 12 h transport time respectively thus only slight increase occurred from 8 to 12 h journey time. Concentration level of lactate in winter increased in a linear proportion as reported in Figure 22a and it was significant ($P<0.001$).

In bulls (winter) change of lactate concentration was in the range of 2.9 – 4.7 mmol L^{-1}, lowest at 4 and highest at 8 h transport time and thereafter declined to 3.5 mmol L^{-1} at 12 h journey time (Figure 22b). The concentration remained unchanged (2.4 mmol L^{-1}) during 4 and 8 h and then increased slightly (2.9 mmol L^{-1}) at 12 h transport time (summer).



(a)

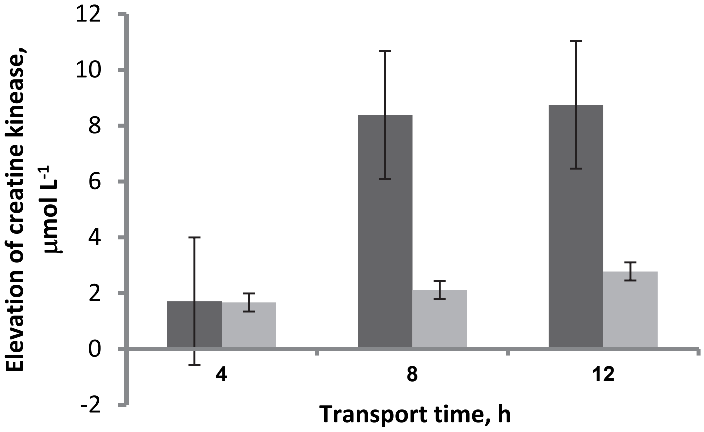


(b)

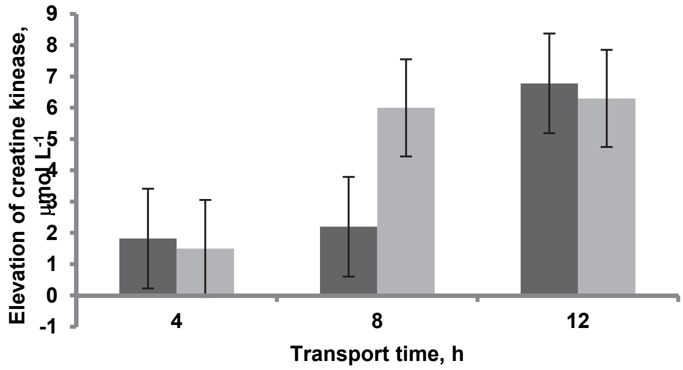
Figure 22. Mean and standard error of lactate concentration level in cows (a) and bulls (b) for different transport times and seasons. Dark and grey colours represent winter and summer respectively.

Creatine Kinease

In cows, both during summer and winter, the concentration of creatine kinase increased with an increase in transport time (from 4 to 8 h) and remained at a steady state or slight increased thereafter (Figure 23a). In winter there is a rapid increase from 4 to 8 h transport time. In bulls, the rate of increase in concentration of creatine kinase was different during winter and summer.



(a)



(b)

Figure 23. Mean and standard error of creatine kinase concentration in cows (a) and bulls (b) for different transport times and seasons. Dark and grey colours represent winter and summer respectively.

There was slight increase from four to eight h but from 8 to 12 h the increase could be as high as three fold. During summer measurement concentration level of creatine kinase increased from four to eight h by about four fold and maintained nearly steady state between eight and 12 h transport time (Figure 23b). In both animal categories, transport had significant effect on creatine kinase concentration ($P<0.001$) and was positively correlated with transport time.

Behavioural observation and quantification

Behaviours like turning, fighting and change of position were related to loose cattle because movement of tied animals was restricted. The total number of observed animals was 162 and 12% of the animals (cows and bulls) were tied. Behaviours of animals were quantified as described earlier and the summary of the results is presented in Tables 11 and 12. Tables 11 and 12 present cumulative frequencies and durations of behaviourial occurrence during loading, transport and unloading.

During loading and unloading, Elimination (El), smelling (Sm), vocalisation (Vc), aggressiveness (Ag), refuse to mount the ramp (Rm) and refuse to go down the ramp (Rd) were the main behaviours of bulls and cows. But reversal (Rv) was particular behaviour of bulls.

During transport, in both bulls and cows, swaying (Sw), restlessness and change of position (Rc), turning (Tn) and loss of balance (Ls) were among the highest scores noted (Tables 11 and 12) and increased with transport time. In both animal categories, swaying, loss of balance and restlessness and change of position were positively correlated with transport time ($P<0.002$).

Table 11. *Cumulative behaviour of cows during 4, 8 and 12 h transport time.*

Acti vity	Behav iour	Transport time, h					
		4		8		12	
		Freq	Freq x Time	Freq	Freq x Time	Freq	Freq x Time
Loading	Ag	0.50	0.16	0.25	0.08	0.67	0.25
	El	0.35	0.13	0.50	0.16	0.47	0.19
	Rm	0.81	0.31	0.75	0.70	0.57	0.51
	SI	0.38	0.06	0.25	0.10	0.53	0.19
	Sm	0.64	0.26	0.50	0.46	0.30	0.24
	Vc	0.54	0.09	0.25	0.06	0.27	0.08
Transport	Ag	1.33	0.06	0	0	0.44	0.02
	El	1.67	0.17	0.33	0.11	1.11	0.11
	Fl	0.33	0.03	0.17	0.02	na	na
	Ft	1	1	2.28	3.13	3	3.96
	Ls	3.33	3.28	3.5	5.55	2.78	12.94
	Rc	3.67	17.27	3.17	28.49	6.67	44.28
	Sm	2	0.6	1.83	1.83	4.56	2.28
	Sw	7	249.09	5.83	296.51	7.56	466.75
	Tn	4	12.5	6	42.96	5	38.15
Unloading	Ag	0.25	0.03	0.25	0.19	0.20	0.03
	El	0.28	0.08	na	na	0.20	0.03
	Ft	0.26	0.08	na	na	0.20	0.04
	Rd	1	0.75	1	2.25	na	na
	Sm	0.46	0.15	0.75	0.21	0.47	0.22
	Vc	na	na	0.25	0.06	0.37	0.15

n (4h) = 27; n (8h) = 31 and n (12h) = 24, where n stands for number of animals

Table 12. *Cumulative behaviour of bulls during 4, 8 and 12 h transport time.*

Acti vity	Behav iour	Transport time, h					
		4		8		12	
		Freq	Freq x Time	Freq	Freq x Time	Freq	Freq x Time
Loading	Ag	1.2	0.2	1.6	0.9	0.67	0.31
	Rm	0.48	3.86	0.25	0.50	0.83	0.49
	Rv	0.30	0.27	0	0	0.33	0.06
	Sm	1.11	0.82	0.08	0.06	0.67	0.29
	Vc	0.58	0.15	0.17	0.12	1.17	0.32
Transport	El	0.5	0.64	0.40	0.55	0.50	0.12
	Fl	na	na	0.30	0.20	0.50	0.06
	Ft	0.75	1.84	1.4	4.41	1.5	5.5
	Ls	1	1.92	2.3	8.72	4	11.32
	Rc	1.5	23.25	3.9	21.35	4.25	82.88
	Sm	1.25	5.5	3.6	12.74	2.75	3.19
	Sw	2	42.25	7.5	179.8	11.5	695.75
	Tn	0.75	4.19	2.30	5.49	1.75	30.91
Unloading	El	0.33	0.12	0.80	0.10	na	na
	Ft	0.29	0.08	0.08	0.19	na	na
	Mf	0.83	0.45	0.08	0.10	1	0.41
	Sm	0.41	0.29	0.08	0.05	0.5	0.17

n (4h) = 30; n(8h) = 26 and n (12h) = 24, where n stands for number of animals
na: not available data

Temperature and relative humidity

The lowest and highest temperatures were recorded in March (-3 ^{0}C) and June (23 ^{0}C) respectively. Transport time (8 h), temperature and relative humidity variations are illustrated in Figure 24. It was an incidence of temperature when it dropped below 0 ^{0}C and varied between -0.5 and -1.5 ^{0}C. During loading the temperature was 12 ^{0}C and it began to decrease immediately after the vehicle started moving.

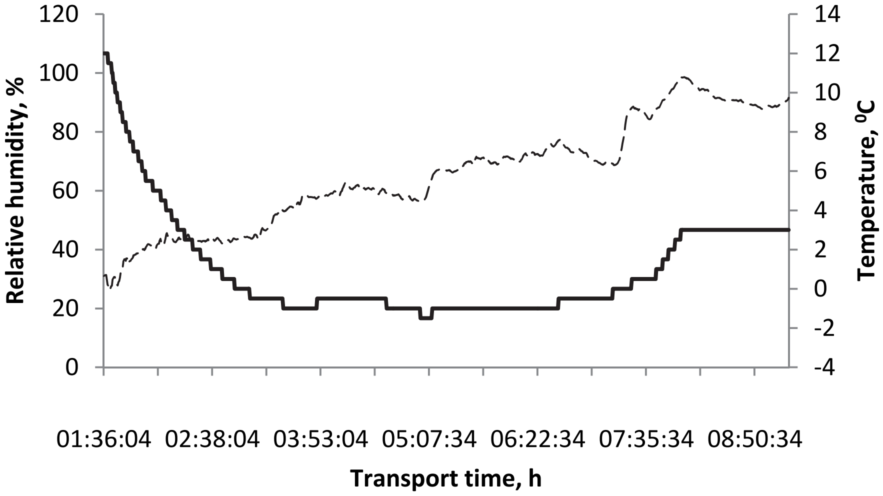


Figure 24. Temperature and relative humidity variations during 8 h transport time. Bold and dashed lines stand for temperature and relative humidity respectively.

It can be observed in the Figure that temperature was already -0.5 ^{0}C after about 1½ h. During the whole trip, temperature variation was between -3 and 12 ^{0}C. It took 4:08 h before the temperature began to rise to zero. Initial relative humidity was 31% at the time of loading and even when the temperature was below 0 ^{0}C relative humidity continued rising from 44 to 74.3%.

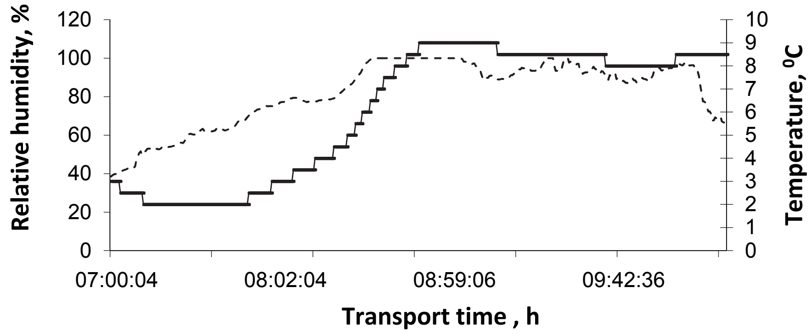


Figure 25. Temperature and relative humidity variations during 4 h transport time. Bold and dashed lines represent temperature and relative humidity respectively.

Initial relative humidity and temperature were 38.5 % and 3 ^{0}C respectively (Figure 25). Relative humidity was rising during loading and transport time and the growth were gradual not rapid growth.

Figure 26 depicts the changes in temperature and relative humidity during 4 h transport time. The initial temperature at the time of loading was -2 ^{0}C and continued falling to -3 ^{0}C during transport. The total time where temperature remained below zero was 2 h and 30 minutes. During loading initial relative humidity was 35%. Despite the decline of temperature for the specified duration relative humidity kept on rising to a level of 70.7%. The relative humidity also continued rising when the temperature started rising and reached up to 75% and thereafter maintained a steady state. During the whole trip temperature varied between -3 and 3^{0}C.

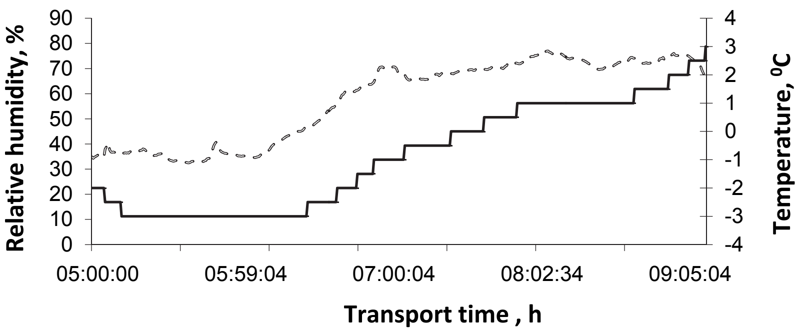


Figure 26. Temperature and relative humidity variations during four h transport time. Bold and dashed lines represent temperature and relative humidity respectively

Multivariate analysis

The General Linear Model (GLM) was used to assess variance using multivariate analysis of variance (MANOVA). Transport time was independent, while blood and behavioural parameters were dependent variables. Considered blood parameters were cortisol (C), lactate (L), glucose (G) and creatine kinase (Ck). Loss of balance (Ls), swaying (Sw), restlessness and change of position (Rc), turning (Tn), fighting (Ft) and elimination (El) were the behaviours considered. Transport, blood hormones and behaviours can be described using a mathematical function that related an input to output variables. In this case transport was an input, blood hormones and behaviours were output variables. Due to transport (input), blood hormones had different values before and after transport similarly behaviours were also different than in the farms. Therefore the main idea of the expression was that the function of transport produced blood hormones and behaviours as output and they were different than before transport and could be expressed as:

$$f(T) = C\ Ck\ L\ G\ Ls\ Sw\ Rc\ Tn\ El\ Ft + \varepsilon$$

The mathematical expression also included standard error, ε so as to be able to run it in SAS programme.

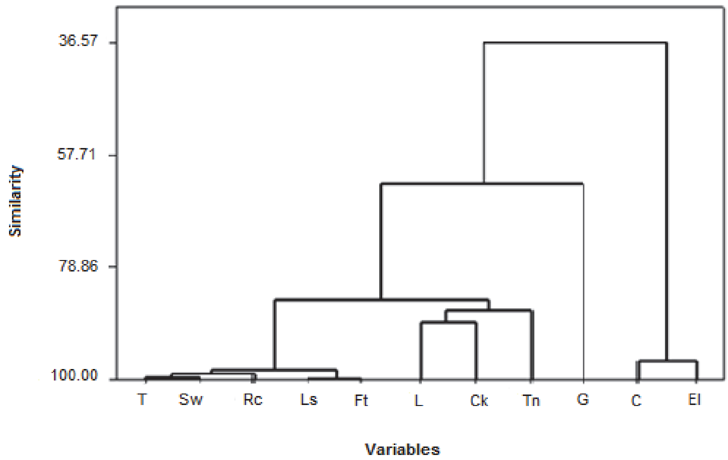
$\varepsilon = n \times p$, and p is a dependent variable. Each of the p can be estimated and tested separately.

Summary of the results are presented in the form of correlation matrices for cows separately (Tables 13). The rows and columns in the Tables correspond to 11 variables.

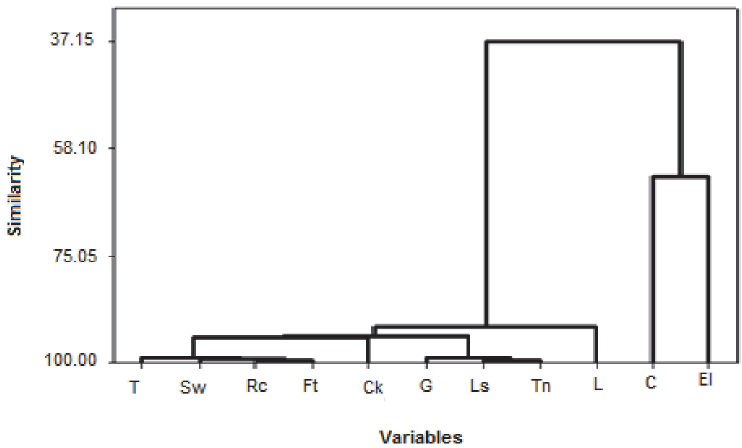
Correlation matrix of variables in cows, as it can be observed in Table 13 that transport time has relatively stronger correlation with behaviours like, loss of balance (Ls), swaying (Sw), restlessness and change of position (Rc), fighting (Ft) and turning (Tn). Lactate (0.7483) and creatine kinase (0.5356) were the blood parameters that demonstrated rather strong or fairly strong correlation whereas glucose (G) was weakly correlated with transport time. Elimination (-0.866) and cortisol (-0.7599) were negatively correlated with transport time. Certain blood hormones and behaviours such as cortisol – elimination (0.8928); lactate – fighting (0.7706) and creatine kinease – restlessness and change of position (0.5081) could also inter correlated at different levels. Moreover there are inter correlation between blood hormones as creatine kinease – lactate (0.6859); creatine kinease – glucose (0.6025) or between behaviours like loss of balance – swaying (0.9235) and restlessness and change of position – fighting (0.9373) and the matrix correlation was significant ($P<0.001$).

Table 13. *Correlation matrix of transport time, blood and behavioural parameters in cows.*

	T	C	L	G	CK	Ls	Sw	Rc	Tn	Ft	El
T	1.0000	-.7599	0.7483	0.1490	0.5356	0.9546	0.9495	0.9943	0.7834	0.9691	-.8660
C	-.7599	1.0000	-.3978	0.4595	-.2190	-.5856	-.7639	-.7056	-.8861	-.8522	0.8928
L	0.7483	-.3978	1.0000	0.5769	0.6859	0.6595	0.6635	0.7245	0.7025	0.7706	-.7402
G	0.1490	0.4595	0.5769	1.0000	0.6025	0.2442	0.0505	0.1846	-.0943	0.0599	0.0421
CK	0.5356	-.2190	0.6859	0.6025	1.0000	0.4429	0.3138	0.5081	0.5623	0.5757	-.5786
Ls	0.9546	-.5856	0.6595	0.2442	0.4429	1.0000	0.9235	0.9809	0.5628	0.8516	-.6778
Sw	0.9495	-.7639	0.6635	0.0505	0.3138	0.9235	1.0000	0.9502	0.7082	0.9059	-.7936
Rc	0.9943	-.7056	0.7245	0.1846	0.5081	0.9809	0.9502	1.0000	0.7128	0.9373	-.8079
Tn	0.7834	-.8861	0.7025	-.0943	0.5623	0.5628	0.7082	0.7128	1.0000	0.9126	-.9892
Ft	0.9691	-.8522	0.7706	0.0599	0.5757	0.8516	0.9059	0.9373	0.9126	1.0000	-.9626
El	-.8660	0.8928	-.7402	0.0421	-.5786	-.6778	-.7936	-.8079	-.9892	-.9626	1.0000



(a)



(b)

Figure 27. Dendrogram of transport time and stress related behaviours and blood parameters of cows (a) and bulls (b).

Dendrogram of transport time, blood hormones and behaviours of cows and bulls is reported in Figure 27. In Figure 27(a) transport time (T), behaviours swaying (Sw), restlessness and change of position (Rc), loss of balance (Ls) and fighting (Ft) clustered in pair form at almost same level of similarity, 97-100%. The level of similarity indicated that the behaviours are strongly

correlated with transport time. Lactate (L) and creatine kinase (CK) could also form a pair wise cluster at around 90 % depicting fairly strong correlation with transport time. Behaviour like turning (Tn) and a blood hormone glucose (G) added to the cluster at around 89% and 62% respectively. It demonstrated that glucose was weakly correlated with transport time. Cortisol (C) and elimination (El) formed a pair and sequentially added to the main clusters at around 36 %, indicating weak level of similarity. Table 13 also provided a good picture on the relationship of cortisol and elimination with transport time.
In Figure 27(b), dendrogram of bulls the clusters are mainly composed of swaying (Sw), restlessness and change of position (Rc) and fighting (Ft) together with transport time. Other variables also formed pair or added to the clusters. In all these cases the level of similarity lies between 90–100%. However cortisol (C) and elimination (El) added to the cluster at nearly 37% same as in the case of cows. Behaviours and blood parameters and their correlation with transport time indicated that transport induced stress to the animals.

5 Discussion

5.1 Logistics chain and animals transport

Un even distribution of vehicles arrival at the abattoir delivery gate led to queuing of unloaded trucks and consisted about 20% of the total number of vehicles. The vehicles should be washed before leaving the abattoir. Queue build up at the delivery also gave rise to another cycle of queuing at vehicle washing and it occurred at about 29% of the trucks. Queuing both at delivery and vehicle washing was idle time for transporters. Thus queuings at the delivery gate and vehicle washing were one of the major problems in terms of animals welfare and unproductive time.

Cattle were frequently kept for more than 1 h and occasionally more than 2 h in the lairage box. During a technical breakdown in the slaughter chain, lairage time for the animals increased to an average of 3.8 h; ranging from 2.2 to 6.5 h. The lairage boxes were narrow in space and the natural movement of the animals was more restricted than during transport. The noise, smell and the environment as a whole were additional stress inducing factors. Hence the lairage time of the animals should be as short as possible to not aggravate their welfare.

In the study, transport time and distance could be reduced through the use of route optimisation, saving up to 23% in time for individual routes and with the overall average of 3.9% both in time and distance. The usual routine is that geographic areas of animals supplying farms were divided into sectors and one transport firm was assigned to each sector. Though the long term plan of the abattoir was based on producers' pre-registration delivery, the detailed transport planning was performed by the transport operators. Route optimisation was performed within the given geographic areas. But route optimization beyond the boundaries of the sectors could have provided better results in terms of welfare, distance, time, cost, and environmental benefit. Furthermore better vehicles arrival distribution at the abattoir could also be achieved.

5.2 Vibration levels on animals, vehicles and related behaviours

Cattle and vibration as related behaviours

During vibration measurement on various road conditions and speeds, the highest values measured on animals were (2.23±0.27 and 2.27±0.33 m s^{-2}) in the driving direction on gravel roads at speeds of 50 and 70 km h^{-1} respectively. Animals experienced lower horizontal vibrations when facing perpendicular to the driving direction than when facing the travel orientation. They were also exposed to lower levels of lateral acceleration in the perpendicular orientation to travel direction. Animal facing in travel direction is exposed to a greater degree of loss of balance as the result of speed variations and braking. In bad road conditions and rough driving speed the risk for injury can be higher compared to animal facing in perpendicular orientation. This work has shown that combinations of road conditions, vehicle speeds and cattle standing orientations have significant influence on the level of vibration.

Swaying and loss of balance were vibration related observed behaviours and could constantly increase in cows and bulls. In bulls, swaying behaviour increased in an exponential but for cows in linear form (Figure 9). Tarrant (1990) reported that during transport of steers, swaying of a truck from side to side could account for loss of balance. It is also found that cumulative effect of swaying and surge during a long road journey were likely to explain the increase in butt and pin bruising with increased road distances (Wythes et al., 1985). Swaying doesn't belong to the common behaviours of cattle in the farm. During transport, swaying occurred due to motion of the vehicle. The animals might sway to maintain balance or involuntary. But as the swaying behaviour increases with transport time it can cause muscle fatigue and welfare can be impaired.

Loss of balance also increased with transport time in a linear form in bulls and in an exponential in cows. In bulls loss of balance increased from 4 to 8 h more than 4 times and at 12 h slightly increased (Figure 10). In cows rapid increase was noted from 8 to 12 h and it raised more than two fold. According to Tarrant (1989 and 1992) loss of balance can be attributed to driver behaviour such as braking and stopping. Poor driving performance and poor suspension system are the main factors causing vibration and loss of balance during animal transport (Gebresenbet, 2003). Roads conditions (poorly maintained road, surface smoothness, potholes, corners, slopes, etc.) and transporters' driving quality are the principal causes for the animals to lose balance. The floor of the vehicle which used to be wet (due to urination and defecation) some hours after transport could also be additional factor for loss of balance. Loss of balance occurred when the animals had difficulties in holding balance and fatigue.

Swaying and loss of balance behaviours can affect welfare of cattle in the form of bruise, injury and fatigue. When loss of balance is considerably serious, cattle may fall and may have difficulty in standing up. Other pen mates may tramp the animal that makes the welfare status severe. Besides, falling of one animal can be a cause for other animals to lose balance and eventually fall.

Regarding cattle vibration data there is lack of reference data to rely on and due to this reason, human vibration data were used to evaluate levels of vibration. The EU vibration directive 2002/44/EC ensures minimum protection requirement for workers exposed to vibration and sets daily exposure action (0.5 m s^{-2}) and daily exposure limit (1.15 m s^{-2}) values. During 8 h transport time vibration exposure values of cattle were related to the daily exposure action and limit levels for human. In that respect, vibration exposure values in vertical (0.61±0.12 m s^{-2}), horizontal (0.92±0.35 m s^{-2}) and lateral (1±0.21 m s^{-2}) directions for duration of 8 h exceeded the EU daily exposure action (0.5 m s^{-2}) but were below the daily exposure limit (1.15 m s^{-2}). All the values were above the safe and fell in the alert region. According to Nosal and Gygax (2005) in dairy farms when vibration and noise exceed 0.2 ms^{-2} and 70 dB the health and yield performance of the animals was affected. Transported cattle were exposed to vibration levels which were regarded as unsafe region for humans.

Transmitted acceleration, Vibration Dose Value (VDV) and root mean square (r.m.s) from floor to animal were 100-158%, 101-154% and 95-143% respectively. When vehicle floor was compared to the driver seat, the seat is equipped with vibration reducing structures and in addition to that the contact between the floor and the animal is not rigid. Moreover, as the upper part of the animal's body also sways in relation to the footing, vibration levels on upper body part of the animals could be higher than on the floor. These were the main reasons for amplifying vibration on the animal.

Pigs and vibration as related to behaviours

Transport of pigs in the observation box was characterised by a lying behaviour. Over 85% of the pigs in the observation pen were lying in the direction of travel. Moreover they had very close body contact and formed group lying and it resulted in swaying in group while lying. The possible explanation of this behaviour was that level of vibration on the 3rd floor was greater in lateral than in horizontal (travel) direction and vibration in lateral direction was not comfortable for lying as compared to horizontal. Around 10% of the pigs were lying perpendicular to the direction of travel but often supported against side walls.

The last feeding time for pigs to be transported was 4 h before the start of loading to avoid travel sickness. Nevertheless, the incidences of travel sickness particularly retching and vomiting behaviours increased in proportion to transport time (Figure 7). It has also been reported by other authors that, heavy feed intake by pigs immediately prior to loading has been associated with vomiting and death loss as they are monogastric animals (Randall & Bradshaw; 1998, Guise, 1987). In journeys with certain vibration characteristics, pigs show behavioural evidence of motion sickness in that they retch and vomit

(Randall & Bradshaw, 1998; Bradshaw et al., 1996). Travel sickness mainly retching and vomiting increased together with transport time and duration of vibration even when the stomach was empty. During travel sickness, the animals displayed other signs of travel sickness as excessive salivation and repeated swallowing prior to retching and vomiting. The lying of pigs in the horizontal direction might be to reduce the incidence of travel sickness. These signs and behaviours do not occur commonly in pigs farm unless the animal is sick. Therefore, vomiting and retching can be regarded as behaviours that occur under stressing environment.

Vibrations on floors of pigs transport vehicle

On pigs transport truck, the ratio of transmitted vibration (using acceleration, r.m.s and VDV) from floor one to floor three was greater than 100% in lateral and vertical direction. But in the horizontal direction it was below 100% therefore dumped only along this direction (Table 5). Variation of vibration levels on floor one and floor three was due to: that floor one was located relatively closer to the suspension system than floor three and dumping system was more effective in this floor. Moreover, floor one was rigid to the chassis of the vehicle. This could be the possible explanation for lower vertical and lateral acceleration on floor one. When floor three was raised to a specific height, it was supported (by mechanically means) along the two sides of the walls at three points on each side. Square formed rubber resiliencies were placed on front and rear sides of the floor to attenuate vibration during forward and reverse speed variations along driving direction. This resulted lower vibration level on the floor in travel direction. Therefore, pigs in floor one were exposed to higher horizontal while those in floor three were exposed for higher lateral and vertical accelerations. The designs of floors two and three were the same, and it is therefore more likely that pigs in the second floor were also exposed to higher lateral and vertical accelerations.

Driving performance

Trips with different drivers could result in various vibration levels despite that the routes and transport time remained the same (Figure 8). Careful driving, especially on bends and corners on route, and during acceleration and braking have a substantial effect on the welfare of cattle (SCAHAW, 2002; Broom, 2008). The way in which animals are loaded, unloaded as well as the way in which a vehicle is driven can have enormous effects on the welfare of transported animals (Broom, 2005). Despite the same vehicle and roads conditions, driving performance of various transporters could result in different levels of vibration in all axes. Therefore welfare of pigs can be affected by driving performance.

5.3 Effect of transport time on pigs and cattle welfare

Pigs

Climatic conditions, THI and pH value

In a naturally ventilated vehicle, when the vehicle was in a stationary position, ventilation was inadequate to reduce heat inside the vehicle. During loading there was a rapid build-up of heat as the number of animals increased sequentially. A combination of this and high initial relative humidity can affect welfare of pigs. During hot season, in terms of heat stress duration of loading has a significant effect on welfare of pigs. The mean values of Thermo Humidity Index (THI) for both winter and summer seasons were below the thresh hold (<74). However at 12 h transport time, 79.2 THI value was noted during loading. Carcass ultimate pH value during summer season for 12 h transport time exceeded 5.8, and reached 5.9. The above threshold and peak THI value inside the vehicle could attribute to the high ultimate pH value.

During field measurements, the lowest temperature (-2 ^{0}C) was recorded in February and the highest (28 ^{0}C) in July and the highest value occurred during loading event. In all measurements, whether temperature was rising or falling, relative humidity constantly increased during transport. When the initial relative humidity was above 60% (Figure16) there could occur a rapid increase and sometimes reached the maximum level during loading. Meanwhile, if the initial relative humidity level was below 60% (Figure 17), there was comparatively slow relative humidity growth and thereafter it decreased rather faster, as the vehicle started moving (compared to higher initial humidity). Inadequate ventilation after washing was the main cause for high initial relative humidity. Moreover during loading, the successive increase of animals and poor air movement raised both temperature and relative humidity.

Blood hormones and behaviours

Cortisol concentration was significantly elevated (P<0.001) during the first hours of transport and the concentration decreased as duration of transport increased. According to Pérez et al. (2002), when a 15 min was compared with 3 h transport duration, pigs subjected to a shorter journey showed a more intense stress response thus increased cortisol and lactate levels. Cortisol is an indicator of stress caused by fear and arousal and can be regarded as an immediate response of the animals.

Glucose concentration increased from 4 to 8 h transport time and thereafter decreased slightly (P<0.01) during winter. The behaviour of glucose concentration during summer was also similar to that of winter but after 8 h it decreased sharply. Mota-Rojas et al., (2009) reported that in pre-slaughter transported pigs glucose showed high increase in blood concentration, associated to an increase in muscular activity. Lactate concentrations (winter) were positively correlated ($P < 0.002$) with transport time and during summer the concentration levels were nearly the same at 4, 8 and 12h transport time. Stress due to physical exertion increased the concentration of lactate (Knowles & Warriss, 2000). When the exercise or muscular activity is not strenuous, glucose takes over and lactate concentration decreases. Thus lactate production is closely related with the intensity of muscle exercise and the concentration is enhanced during strenuous activity of the muscles.

Creatine kinase concentration was significant ($P<0.002$) and increased in an exponential form with transport time in both winter and summer seasons. The rate of increase from 8 to 12 h was about 5 fold (winter) and 3 fold (summer). Creatine kinase is an enzyme found in muscle tissue. The damage to muscle cells resulting from the strain inflicted on animals during pre-slaughter handling releases the enzyme into the bloodstream. Elevated levels of creatine kinase activity are indicative of the extent of tissue damage (Harris, 1993). The release and increase of creatine kinease concentration in the blood is not only when there is muscle damage, it also leaks in the blood as the result of vigorous exercise and muscle activity. The continuous increase in creatine kinase with an increase in transport time noted in the study indicated increasing muscular fatigue, which could be attributed to restlessness, loss of balance and fighting during transport. There was constant growth of loss of balance with journey duration at 4h (0.21), 8h (0.43) and 12h (0.94) (Table 7) transport time.

In cluster analysis, behaviours like lying, panting, restlessness and change of position together with blood parameters demonstrated stronger level of similarity to transport time. The correlation with the exception of lying indicated that animals were under stressful condition during transport.

During loading, pigs that reached the slaughter weight were selected from several pens and loaded in a mixed form. Mixing with unfamiliar animals led to fighting. This became an introduction to a new social order which eventually led to fighting. In this measurement fighting (Ft) increased from 4 h (0.16) to 8 h (0.21) and maintained almost steady state at 12 h (0.23) transports time.

Pigs relied mostly on panting to dissipate heat from the body. During transport panting was the only available means to regulate body temperature. The rate of panting (Pt) increased with duration of journey after 4h (0.02) linearly at 8 (0.55) and 12 h (0.86) transport time. According to Robertshaw (2004), panting and sweating account for 20% each and wallowing for about 60% of the relative proportion of total evaporative heat loss in swine. Under transport conditions, pigs can lose only small percent of heat and for this reason it is extremely important to maintain temperature and relative humidity below the thresh hold limits.

Rooting occurred highest at 12 h (15.67) and lowest at 4h (3.68) and the rate of growth from 8 (4.72) to 12h (15.67) was higher than from 4 to 8h transport time. According to Studntiz et al. (2006) pigs explore their surroundings by rooting, sniffing, biting and chewing various food items as well as indigestible items. In this way pigs become familiar with their environment and the various resources within it. Smelling demonstrated growing trend from 4 (2.33) to 8 h

(4.17) and at 12 h (4.29) maintained almost same level as the value of 8h transport time. Olfactory of pigs are well developed and match those of dogs. Pigs use their sense of smell to search for and examine food and to recognise group members (McLeman et al., 2008; Spinka, 2009). Restlessness and change of position (Rc) also raised from 2.29 (4h) to 3.62 (8h) and 3.67 (12h) as duration of transport time increased. During rail transport pigs that travelled from England to France and thence to Italy over seven days were only restless when hungry or thirsty (Jackson, 1979). The animals were deprived from feed 4 h before the start of loading to reduce the risk of travel sickness and vomiting. As transport duration increased the need for food and water tended to grow. It is more likely that the growing occurrence of rooting (Rt), smelling (Sm), restlessness and change of position (Rc) behaviours were the effects of lack of feed, water. Other stressing factors inside the vehicle like climatic conditions, motion of the vehicle and interactions within the animals etc. were also responsible for these behaviours.

During transport roughly after two hours the number of lying (Ly) pigs measured in frequency minute increased constantly at 4 h (62.63), 8 h (812) and 12 h (1269.35) transport time. Sitting (St) and lying one on another (Ln) could also increase with transport time but at different rates. Lying behaviour of the animals resembled the behaviour they exhibited in the farms. According to Ekkel et al., (2003) about 75% of the time pigs are lying. Pigs have two peaks of activity, one in the early morning, and the other in the evening. They are usually found resting once dusk has fallen (Compassion in World Farming Trust, (CIWF), 2006). Sitting and lying one on another might occurred due to limited space. If transport conditions are inconvenient for pigs to lie and forced standing may easily lead to fatigue and exhaustion.

It can be concluded that lying, sitting, rooting, vocalization, panting, fighting, loss of balance, and restlessness and change of position had strong level of similarity with transport time.

In nearly all the measurements concentration levels of blood hormones after transport were higher than the control values. The blood parameters demonstrated varying relationship with transport time. Glucose was weakly correlated but the relationship of lactate was linear with transport time. The strongest relationship was noted between transport time and creatine kinease. But most importantly the rate of increase was considerably greater from 8 to 12 h by more than 5 fold (winter) and by about 3 fold (summer). This indicated that intensity of physical activities decided the level of creatine kinease activity. Though thresh hold values are lacking to evaluate the animals level of stress during transport. Based on lactate, unique development of creatine kinease activity and the constantly increase of stress indictating behaviours it can be concluded that transport from 8 -12 h had a higher effect on welfare of pigs.

Cattle

Loading and unloading

Loading procedures depends on keeping method at the farm, whether loose or tied. In comparison to loose, it required more time to load tied cows (3.34±1.74 versus 2.73±1.57 min) and bulls (6.5±3.7 versus 0.77±0.49 min). Moreover to load tied bulls (6.5±3.7 min) it demanded more time than tied (3.43±1.74 min) cows. Loading tied animals mainly bulls have to be accomplished by experienced transporters, as it may be risky to both humans and animals when it is performed by inexperienced transporters. Cattle from tied housing systems are more stressed by transport than untied cattle and there is a greater risk to develop bad carcass- and meat quality (Gebresenbet et al., 2005). Loading and unloading tied, in relation to loose animals not only it required more time and highly experienced transporters, it was also more stressful for the animals. Even during transport movement of tied animals was more restricted than loose ones.

pH value

The most common results observed in the study were within the range of minimum and maximum threshold. Some values were below the minimum threshold value of 5.4 in cows and bulls. For the current case, it was probably due to the temperature in the samples. The values between 5.8 and 6 are considered to be of lower meat quality but are not yet classified as DFD meat (Nguyen et al., 2006). The final pH_{24} values for both winter and summer ranged from 5.58 to 5.28 in cows and 5.60 to 5.34 in bulls. Thus transport time has shown any significant effect on pH_{24} values in any animal category.

Temperature and relative humidity

High initial relative humidity contributed most to rapid increase in humidity, together with rising temperature whereas when the initial relative humidity did not exceed 40%, the growth was gradual and remained mostly around 80 – 90% regardless length of transport time. The possible reason for high initial humidity (above 40%) could be the availability of high moisture after washing the vehicle and insufficiently ventilated floors and side walls. In those cases where the values were below 40% prior to loading the rise of relative humidity was not rapid but gradual (Figure 25).

Temperature dropped below 0 ^{0}C or below the recommended level (up to -3 ^{0}C) during certain field measurements (Figure 24 and 26). During these trips, temperature remained below zero for 4:08 and 2:30 h of the journeys duration. Nevertheless relative humidity was increasing even during those incidences to various levels and reached 74%.

When the amount of moisture in the atmosphere does not change, there exist an inverse relationship between temperature and relative humidity. If the temperature increases, relative humidity decreases and vice versa (Valsson & Bharat, 2011). In cattle transport vehicle, temperature inside the vehicle is not the sole factor influencing relative humidity. Animals in the vehicle produce heat from metabolism and moisture from respiration, urination and defecation. It can further be dictated by the number and size of the animals. The exchange of heat between the animals and the vehicle container is a continuous process. Therefore, when temperature was below 0 ^{0}C there was still moisture or water vapour inside the vehicle resulted from heat exchange, respiration, urination and defecation. But the rate of increase was small as compared with temperatures above 0 ^{0}C.

Low temperatures and high air humidity may enhance heat dissipation and the animals suffer from cold (EC, 1999). The recommended temperature and relative humidity values by EFSA (2004) included upper limits for temperature and relative humidity and lower limits only for temperature. But when temperature is falling and relative humidity is rising, welfare of animals is impaired not only because of temperature but also of the combined effect of temperature and relative humidity.

Table 14. *Recommended minimum and maximum temperature EFSA Scientific Panel (2004).*

Age of cattle in weeks	Min temp ^{0}C	Max temp adjusted for humidity ^{o}C	
		RH < 80%	RH > 80%
0 - 2	10	30	27
2 - 26	5	30	27
> 26	0	30	27

Therefore low temperature (below 0 ^{0}C) and high humidity affect welfare of cattle even though only temperature limit is specified in the recommended Table (Table 14). The main causes of temperature below the recommended level were that when outside temperature was below 0 ^{0}C and the container was poorly insulated. It could also occur due to unnecessarily delayed loading time and as the door container remained open.

Blood hormones

In winter and summer seasons cortisol level in cows decreased with an increase of transport time. Similar results have been reported by previous researchers. Lay *et al*., (1996) found that cortisol concentrations in cattle decreased as a result of habituation during prolonged or repeated exposure to transportation. Warriss et al. (1995) also investigated the effect of transport time on cattle for five, 10 and 15 h, and concluded that cortisol concentration level decreased with transport time. In older cattle, cortisol concentrations increased in response to loading, unloading, and during the first portion of a journey (Wariss et al 1995; Grandin 1997; Knowles et al., 1999). Lay et al., (1992a, 1992b) also reported that, beef cattle not accustomed to handling had significantly higher cortisol levels after restraint compared with dairy cattle that were accustomed to handling. Bulls and some of the cows were transported once in their early stage. Transport was not part of the animals regular activity and it consisted of handling, leaving the original place and groups, mounting the ramp and placing in a limited space. Transport and associated conditions were therefore new to the animals. The main reason for the rise of cortisol concentration could strongly be related with initial attempt of the animals to cope with and habituate the new environment. Nevertheless concentration level of the hormone declined as transport time increased from 4 to 8 and 12 h. Cortisol concentration level in bulls behaved in similar way as in cows except for 12 h summer transport time, it could not fall with transport time. Most of the bulls were raised for beef and they were only 18 months old and didn't have same level of exposure to human as the dairy cows. This could be the reason for high level of cortisol concentration in bulls at 12 h transport time. In cows, concentration level of glucose was lowest at 8 and highest at 12 h transport time. Glucose concentration level of bulls increased with the increase in transport time. According to Cole et al. (1988) physiological measures including serum glucose and creatine ratio were affected by transport duration in a linear fashion. When an animal is transported, psychological, physiological, and physical stress result in the release of hormones that correspond to elevated blood glucose concentrations (Kannan et al. 2000).

In cows the highest concentration level of glucose at 12 h transport time (after transport) was 7.53 mmol L^{-1} and the mean reference value in control cows was 5.4 mmol L^{-1}. In the case of bulls 9.2 mmol L^{-1} was the maximum noted glucose level during 12 h transport time (after transport) and the reference value was 4.7 mmol L^{-1}. The corresponding reference value in the literature (Doornenbal et al., 1988) is 4.4 mmol L^{-1}.

In cows concentration level of lactate increased almost in a linear form against transport time. Highest lactate concentration level of cows was noted at four and 12 h winter transport time (11.6 mmol L^{-1}) and it was higher than the control value (1.2 mmol L^{-1}) by more than 9 folds.

During winter lactate elevation in bulls was highest at 8 h and lowest at 4 h transport time. In bulls 15.2 mmol L^{-1} was the highest lactate concentration at 12 h summer transport time whereas the control value was 3 mmol L^{-1}. Acute exposure of cattle to adverse stressors causes release of catecholamines which also stimulate glycogenolysis, leading to mobilization muscle glycogen stores, together with elevated blood glucose and lactate concentrations (Knowles & Warriss, 2007).

During winter experiment change in glucose concentration (cows) was not in consistent with transport time. It was lowest and highest at 8 and 12 h transport time respectively. On the other hand lactate growth in concentration was in consistent with transport time, exhibited a linear growth. Instead of glucose it was more likely that lactate level started to increase when there was acute energy demand of the body muscles as a result of increasing stress.

In bulls (winter) highest and lowest concentration level of lactate was during 8 and 4 h transport time but in summer the level of concentration was the same at 4 and 8 h journey and it was highest at 12 h trip. During winter and summer measurements glucose concentration could rise linearly as transport time increased. Under relatively stable stress condition, the energy requirement could be covered by growing glucose concentration. This could be the reason for lower lactate level at 12 h transport time. Moreover in both animals categories the low glucose or lactate level could further being compensated by the growing level of creatine kinase concentration.

During winter, in bulls at 8 h transport time, highest creatine kinase concentration (140 µmol L^{-1}) was measured and it exceeded the control value (87.5 µmol L^{-1}). In cows 196 µmol L^{-1} was the highest level of creatine kinase concentration measured during winter at 8 h transport time and it was higher than the control value (122 µmol L^{-1}). It is reported that plasma levels of creatine kinase not only increase proportionately with the duration of the journey, but they also remain high for several days after transport (Warriss *et al.* 1995, Knowles *et al.* 1999).

The concentration level of creatine kinase in bulls and cows correlated with transport time during both winter and summer. In both animal categories the growth of concentration in creatine kinease was sharp from 4 to 8 h journey time and thereafter maintained almost steady state or increased slightly between 8 and 12 h (Figure 23 a and b). The enzyme creatine kinase is released into the blood stream when there is muscle damage and intensive muscular activity. However during the experiments there were no injuries or muscle damage and the high level of concentration was therefore related to muscular fatigue resulted from vigorous exercise of the animals.

Behaviours, blood hormones and their interactions

During loading the stress indicating observed behaviours (bulls and cows) were elimination (El), smelling (Sm), vocalization (Vc), aggressiveness (Ag) and refuse to mount the ramp (Rm). Refusing to mount the ramp had the highest score in both animals categories, reflecting high stress level. At the beginning of a journey, cattle are generally anxious and restless and defecate and urinate frequently (Knowles et al., 1999).

When the animals were in transit swaying (Sw), restlessness and change of position (Rc), turning (Tn), loss of balance (Ls) and fighting (Ft) behaviours could exhibit growth with transport time (Table 11 and 12) and elimination

(El) was highest only at 4 h in both cows and bulls. This could further be indicated in correlation matrix (Table 13) and Figures of dendrograms (Figure 27a and b). In the Table of correlation matrix these behaviours were strongly correlated with transport time. The Table displayed not only the correlation of these behaviours with transport time it also included inter correlation between blood hormones and behaviours. Cortisol and elimination were negatively correlated with transport time, and but they have strong inter-correlation because the main reason for cortisol elevation was fear and arousal, even frequency of elimination (defecation and urination) was influenced by a similar situation. According to Ekesbo (2011) if cattle are nervous or frightened they defecate more often and usually more liquid faeces. Gruber at al., (2010) also reported interaction between blood hormones and behaviours of cattle. When cattle exhibit adverse behavioural reactions to handling, values for plasma epinephrine concentration, heart rate, and rectal temperature increased. Færevik et al., (2006) also reported that, isolation of cattle induces struggling, vocalization, increased heart rate and plasma cortisol levels. During fighting the animals were under strenuous exercise which also raised muscle energy requirement. This could be the reason for the inter correlation of fighting and lactate. In relation to blood hormones and behaviour Gruber at al., (2010) further reported that cattle during chute restraint at the slaughter had, elevated plasma lactate concentration.

Swaying, restlessness and change of position, loss of balance and fighting behaviours that occurred during transport differed from the common behaviours of the animals in the farms. Feeding, drinking, resting and lying are some of the basic behaviours of cattle in farm environment. It is found that adult cattle rest lying for 10.1-11.6 h of the 24 cycles (Krohn & Munksgaard, 1993). The finding is further enhanced by Jensen et al., (2005) who express that cattle show a strong motivation to rest in a lying position. They are inclined to invest a lot of effort in order to be able to lie down. However, lying time will be reduced if the lying surface is dirty or damp (Keys et al., 1976).

In all the journeys despite the lying behaviour, the animals were transported only in standing position thus deprived of resting and lying. Involuntarily prolonged standing and swaying could eventually induce fatigue, loss of balance and restlessness. In addition to that transport was performed without feed and water. Therefore all these combined with other transport conditions could exacerbate the stressing situation of the animals.

Stress indicating behaviours were persistent with duration of transport. The blood parameters correlated with transport time in varying levels but the enzyme creatine kinease not only correlated with transport time it also demonstrated a quick growth from short (4 h) to medium (8 h) and maintained almost the same level. Therefore on the basis of behaviours and blood parameters in particular creatine kinease transport from 4 to 8 h had a higher effect than an increase from 8 to 12 h on welfare of cattle.

6 Conclusions and remarks

In Sweden animals were transported under a wide range of climatic variations during the hot and cold seasons of the year and in particular pigs and cattle were exposed to heat and cold stress respectively. In naturally ventilated pigs transport vehicle, there should be adequate ventilation after washing and before the start of loading animals. Pigs loading time which was the main cause of heat stress could be minimised by improved routines. When transport of pigs is conducted during hot season of a year, it is recommended that loading be achieved during cold hours of the day.

Loading, driving, unloading, queuing and waiting in the lairage at the abattoir were the main components of transport and they were also sources of stressful situations. Loading tied animals mainly bulls have to be accomplished by experienced transporters so as to avoid injury to humans and animals. In cattle farms to minimise transport related stress it is highly recommended to apply loose keeping methods.

Ethological parameters, stress hormones, carcass pH value and environmental conditions in a combined form could provide a better picture of animals' welfare status during transport. Fighting among pigs (in transit) can be minimised if separation from original and mixing with new groups could be performed about one week ahead before transport.

In a multi-floor pigs transport vehicle, owing to its design pigs were exposed to different levels of vibration. Those in the third floor preferred to lie in travel orientation. The standing orientation perpendicular to the driving direction is recommended for cattle in terms of vibration level. Animal transporters have to adapt speeds of vehicles to road conditions and conditions so as to minimise the impact of vibration on their welfare. Regular training is one of the key factors to improve driving performance of animal transporters.

Animals transport vehicles have to be equipped with necessary devices to monitor inside climatic conditions and behaviour of the animals.

Shorter transport distance and duration can be achieved by effective planning and route optimisation beyond the geographic boundaries that base on animals species and daily capacity of the abattoir. Moreover, arrival at delivery gate and vehicle washing can be evenly distributed thus reducing stress for the animals and eliminating idle time for transporters.

The overall conclusion from the study of cattle and pigs' welfare and transport time, based on climatic conditions, animal behaviour, stress hormones, and final pH values in carcases, that an increase from 4 to 8 h transport time had higher effect on cattle welfare and subsequent meat quality. But in pigs the increase from 8 to 12 h had a higher effect on welfare.

7 Further research

Behaviours of farm animals are generally reflection of a changing environment and without causing additional stress they can provide sufficient stress associated information. In a view to develop further understanding and to apply in evaluating animals welfare further behavioural investigation will be required.

Stress indicating blood hormones provide information when the animal is at resting state. Threshold values are lacking in a stressful situation and further research is required in this area.

Vibration causes discomfort to farm animals but little is known about the extent of discomfort or welfare level. Due to inadequate data human comfort level are used to evaluate the effect of vibration. It is very important to conduct research on vibration that enables evaluating comfort level of farm animals.

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