Effects of Handling on Animals Welfare during Transport

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Abstract

Transportation is a very complex event which compromises animal welfare and meat quality. Even though important research works have been reported related to animal transport and welfare, many questions are still remaining to be addressed, particularly on the effect of transport time, vibration and climatic conditions.

The main objective of this thesis was to investigate the effects of handling and transport on animals' welfare. The main methodologies employed comprehensive field measurement to collect data, observations, video filming for behavioural study and modeling. The thesis is structured into 3 papers. Paper I focuses on the modelling the dynamic performance of heart rate during loading for transport. Paper II and III are dealing with vibration and effect of transport on welfare of cattle and pigs' respectively.

The model developed to describe the heart rate performances fits and successfully simulates the heart rate. The heart rate increased rapidly from resting value to peak value and then remains under stress, partially under stress or fully (100%) recovered.

The speed, road conditions and standing orientations of animals had effect on level of vibrations. In all road types and speeds, standing orientation of animals facing in driving direction generally exposed to higher horizontal and lateral vibrations than animal facing perpendicular to the driving direction. The three common resonance frequencies identified were 1.3, 5.1, and 12.6 Hz in the second peak at 23Hz in vertical direction on tarmac road at the speed of 85km/h.

Cortisol concentration level elevated during short transport time and decreased with an increase of transport time. Highest and lowest glucose concentrations for winter and summer were at 8 h and 12 h transport time respectively. Concentrations of lactate and creatine kinase positively correlated with transport time. The pH_{24} values correlated with transport time during summer. Behaviours such as lying, sitting, rooting, smelling, panting, fighting, restlessness, change of position and vocalization correlated with transport time.

The overall conclusion from the study was that transport and handling had significant effects on animal welfare. The model developed described well the dynamic response of heart rate of the animals during loading for transport.

Key words: animal welfare, Transport time, behaviour, handling, Stress parameters, Heart rate, Vibration, dynamic response, road conditions.

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Dedication

I dedicated this work to my mother Sobbooqee Haatawuu Gibee and the memory of my father Damisee Bulittaa Boloqee Bokkuu.

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List of Publications

This thesis is based on the following papers, which are referred to in the text by the relevant Roman numeral.

I: Bulitta FS, Bosona TG, Gebresenbet G. 2011. Modeling the dynamic response of cattle heart rate during loading for transport Australian journal of agricultural engineering AJAE 2(3):66-73.

II: Girma Gebresenbet; Samuel Aradom; Fufa S. Bulitta, Eva Hjerpe. 2011.Vibration Levels and frequencies on Vehicle and Animals during Transport.Bio System Engineering, volume 110, pages 10 - 19.

III: Samuel Aradom; Girma Gebresenbet; Fufa S. Bulitta; Musa Adam. 2012.Effects of transport times on welfare of PigsJournal of agricultural science and technology (Publication in progress)

The contribution of Fufa Sorri Bulitta in each research papers included in this thesis was as follows:

Paper I: Model development, analysis of data and writing of the paper together with other co-authors

Paper II: Contributed in data analysis and writing part of the paper together with other co-authors

Paper III: Contributed in data analysis and writing the paper together with other co-authors

Abbreviations

A_1	Rising amplitude
A_2	Falling Amplitude
A_3	The difference between rising and falling amplitude
CF	Crest factor
DFD	Dark Firm Dry meat
eVDV	Estimated Vibration Dose Value
HR _{rest}	Heart rate at rest
HR _{rec}	Heart rate during recovery
HR _{max}	Maximum heart rate
PSE	Pale, Soft, Exudative
PSD	Power spectral density
r_1	Rising rate
r_2	Falling rate
r.m.s.	Root mean square
T_1	Rising time
T_2	Falling time
VDV	Vibration Dose Value

1. Introduction

1.1. Background

Animals are transported for different reasons such as for marketing, slaughtering, fattening and breeding. During transport animals are exposed to different stress inducing factors such as vibration, physical fatigue, injury, noise, high temperature and relative humidity. Just as humans animals feel pain, uncomforted and suffering during poor way of handling and transport. The EU have developed various directives and legislations to improve animal welfare during transport and the current European legislation (EU, 2005) on the welfare of animals during transport helps animal transporters as guidance for transporting farm animals in a safe way from farm to abattoirs or other destinations. The EU (2005) outlines the level of risk related to various aspects of animal transport such as means of transport, transport processes and space allowances of animals.

Welfare of animals could be improved if animal producers, handlers and transporters improve their knowledge on animal perceptions when being in different environmental situations. Animal welfare has been defined in reference to the adaptation ability of animals to cope with changes in new environmental conditions (Broom D. M. (2000)). Stress response parameters' such as stress hormones, heart rate and behavioural alteration are important parameters in describing animals' situations during transport. Generally ensuring animal welfare is a human responsibility which includes consideration in all aspects of animal welfare, including proper management, feeding, watering, housing, disease prevention and treatment, responsible care and humane handling. Pigs' easily feel heat stress that affects their welfare, which can be easily prevented by providing proper ventilation.

The stress caused by handling and transport is induced to animals in different ways. In Figure 1, issues of stress were discussed and some of the effects noted under different situations including transport. The stressful situation can cause psychological and physiological disturbances.

External factors may stimulate animals and animals may perceive as positive and this may result in good welfare and better meat quality. However, if animals perceive as negative it will lead to poor welfare and subsequent poor meat quality. Stress before slaughter can cause undesirable effects on the end quality of meat, such as pale, soft, exudative (PSE) meat and dark firm dry (DFD) meat as a result of poor welfare.



Figure 1. Stress and welfare conditions of animals

The effect of stress on animal's body causes the body to release stress hormones. These chemicals cause heart rate and respiration rate to speed up, and suppress the immune system. There is a variety of environmental conditions which can cause stress in animals. Several scenarios are considered in Figure 1 which were all related to vibration and transport caused stresses such as loading, unloading, injuries, transport time, vibration, poor handling, sickness, noise, novelty, social regrouping, climatic factors, pre-transport management and food and water deprivation. The stress response parameters such as blood parameters (cortisol, lactate, glucose and creatine kinase), heart rate and behavioural changes indicate the level of stress created and how the welfare of an animal is affected.

When animals are stressed, the heart rate increases from its resting condition to its peak value and decreased up to its recovery level. If the animals adapted to the new condition and recovered, the heart rate decreased to the level of resting condition (see Figure 2), if not adapted, animals remain under stress condition showed the level of stress fully or moderately stressful condition as shown on Figure 2 (Gebresenbet et al., 2006).



Figure 2. Animal heart rate variation due to induced stress

Transport time and vibration are among the most important parameters to be considered to improve animal welfare during transport. Even though important research results have been reported earlier, thresholds of vibration levels and transport time for the improvement of animal welfare remain un- answered. Vibration has impact on the animals' health, comfort, and postural stability. It is important to note that most of the previous researchers have not used stress hormones and behavioural parameters to obtain a comprehensive understanding.

The main research questions in relation to handling, vibration and transport time were whether:

- The heart rate is a relevant stress response parameter to measure the stress level of the animal during loading for transport and whether the simulation analysis useful to understand the dynamic nature of heart rate during loading cattle for transport;
- The vibration levels on chassis, floor and animals that developed during transport; and
- > The lengths of transport time have effect on pig's welfare.

1.2. Literature review

Animal responses in terms of physiological and behavioural alteration are indicators of stress levels induced by stressors (G. Gebresenbet and K. Sälvik (2006)). In the assessment of welfare there is important way of adapting to unknown environment for animals and selecting less pressure that makes free from disturbances, resulting for major consequences of the changes in behavior (Broom, 2006). Transport of animals is increasing steadily in recent decades, both in national and international levels, in relation to structural adjustment, specialization of production systems, internationalization and globalization of marketing system (Gebresenbet, 1998). Now a day there were many comments about animal transport that needs more attentions to fulfill transport facilities in national and international levels based on the wittiness of scientific research works, workshops and seminars.

According to Grandin (1978) cattle are transported by road, rail, sea and air for the purpose of breeding, fattening and slaughtering. Rough handling or poorly designed transport conditions determines both animal welfare and meat quality. The important points to be considered is the species of animal transported, age of the animal, means of transport, transport conditions and duration of the journey as well as other factors influencing the welfare of animal. Research has shown that during transportation of pigs the concentration of some blood parameters such as creatine kinase activities exhibited a sharp indication after 1 h which was maintained at 2 h of transportation (Yu et al., 2009). During transport of animals by vehicle, the floor material of the vehicle must be nonslippery, cleanable, disinfect able, sufficiently drained or free from urine, water to reduce injury and number of falling of animals (Gebresenbet et. al, 2010). To accurately assess an animal's reaction, a combination of behavioral and physiological measurements will provide the best overall measurement of animal discomfort (Grandin, 1997). Trunkfield and Broom (1990), discussed in detail that transport has an adverse effect on the welfare of animals. The authors provided the evidences of the changes in heart rate, mortality rate, enzyme, meat quality and the behavioral changes. According to Lambooij et.al (1993) transport can induce stress in pigs. Climatic conditions, loading density, duration of transport, cold draughts, heat stress, social stress, vibrations and noise all affect the condition of the pigs during transport. Pigs are easily affected animal during transport because they need special care and managements.

Now a day animal welfare problems gets great attention and scientific research activities are increasing. In general, as indicated in many different research work, stress is cumulative responses of animal to its surroundings which may result in sever physiological and psychological effects. Animals experience stress for a variety of reasons, feel pain and the emotions. Stress is adaptive to a certain degree, but above those levels it doesn't adapt and animal stays under stress condition (Gebresenbet et.al, 2010). The strength of stress can be

evaluated using stress response parameters, heart rate and animal behavior. In vehicles, during transport, adequate ventilation and protection from temperature extremes (very high or low) are very important for reduction of poor welfare. The animal stress reaction to handling procedure like transportation depends on three important factors: genetics, individual differences and previous experiences of animals (Grandin 1997). According to the Grandin (1997) report, facility design can have a strong influence on experience and poor design is the main cause to increase stress. In all transport processes animals were exposed to environmental stresses such as heat, cold, humidity, noise, motion and other stresses caused by social regrouping. According to Gebresenbet et al., (2005), transport preceding conditions and processes such as preparation, planning, loading, management and unloading at the end of the transport chain needs improvement to enhance the welfare of animals and meat quality.

1.2.1. Transport Time

The process of transport and length of transport time played an important role in animals' welfare in creating or not creating hazardous situation. Transportation causes stress to cattle that may alter physiological variables, with a negative impact on production and health (Murata et al., 1991). Tadich et al. (2005) also found a high creatine kinase value upon arrival at the abattoir after a transport of 16 hours, but without any further increase during 24 h of lairage time. Knowles et al. (1999) noted that after transport of steer and heifers for up to 31 hours, the cortisol concentration continued to increase after the journey and reached a peak after 12 h and then decreased steadily. Plasma cortisol was greatly elevated with onset of transportation of up to 24 hours (Buckham Sporer et al., 2008). This author also reported cortisol concentration reached its peak value at 14.25 hours before returning to the basal concentration at 24 and 48 hours. Wikner et al (2003) reported the effects of various climatic conditions, stocking density and transport times. To transport animals in a safe way and within appropriate time it needs the attention and follow-up of transporter of animals.

In general the effect of length of transport time and its effect on pigs' welfare have not been studied in detail and needs further investigation.

1.2.2. Vibration

The discomfort created by vibration transmission on animal during transport by vehicle increased with the length of exposure time. The vehicles motion and vibration are known to have effects on health, comfort and postural stability of animals. Gebresenbet and Eriksson (1998) also performed comprehensive field measurement on tri-axial vibration using a commercial animal transport vehicle, taking into consideration road conditions and speed variations. The authors reported that the most dominant frequencies identified were 2, 4, 8 and 12 Hz. However, the above investigations were made on relatively good

(asphalted) roads, and vibration levels have been determined for gravelled and curved roads. Animal responses to mechanical oscillation depend on the frequency, magnitude and duration of oscillation. Body response can be highly dependent on frequency variation, so it is usually necessary to indicate the frequency content of vibration (Griffin, 1990). According to ISO 2631-1, the manner in which vibration affects comfort depends on the vibration frequency (spectral) analysis is a procedure for determining the frequency distribution of power (or energy) of a signal, i.e. the power spectral density distribution in a given frequency band (Buzdugan, Miha` ilescu, & Rades, 1986, Chap. 2).

Transportation is a very complex event from the animal's point of view. According to Graham B. (1994), the fundamental frequency of poultry transporters is between 1 and 2 Hz, with a secondary peak of 10 Hz and a chassis vibration in the lateral axis of 12–18 Hz. The author reported that Standing birds maintain stability by wing extension and by flapping or squatting. Vibration and its consequence of motion during transport, affect the welfare of animals. Involuntary muscle and cardiac muscle can be affected by vibration with blood circulation, heart beat and possibly gut control changes as a result (Scott Graham B. 1994). A number of attempts have been made to connect vibration parameters (displacement, velocity and acceleration) with observed animal disturbances of sensitive organs. Vibration is a stress inducing factor on animals as it emanates from the structure of the vehicle, road conditions, vehicle speed, and driving performance of the driver. When high level of vibration is transmitted to animal body during transport it causes muscular fatigue and disturbances.

According to the report of Perremans et al., (1996), heart rate measurements during vibration were dependent on body weight. Perreman (1996) also reported the root mean square acceleration had a significant effect on maximum heart rate. As indicated in the result of current study, during transport, vibration has an effect on the behavioural and emotional conditions of the animal above certain level. Road type can affect the level of vibration, which in turn may affect animals' welfare. According to Kenny and Tarrant (1987c) report the loss of balance on a moving truck was associated with special driving events. During transport, particularly on rough-surfaced roads, the transmission of the vehicle's floor vibration to the animals can be significant and can create uncomfortable conditions by causing the displacement of centre of gravity of an animal, resulting in body disturbance (Randall, 1992; Randall et al., 1995).

Research showed that degree of comfort and levels of vibration experienced by animals during transport by standing on the vibrating floor of vehicles made them swaying and resulted in loss of balance. These indicate that transport can be considered as an acute stressor, causing physiological and behavioural stress in animals. In general, the effect of vibration on animal still has not been studied in detail and many questions without answer.

1.2.3. Heart rate modelling

A model of cattle heart rate during loading for transport has been developed in current study (paper I). The model contains three parameters (heart rate at rest (HR_{rest}) , at maximum or peak (HR_{max}) and heart rate during recovery (HR_{rec}) . The result showed that heart rate is the indication of physical and emotional stress. Many studies conclude that loading of animals at farms, transport on rough and curved roads, variation of vehicle's speed, mixing of animals with un-familiar animals from other farms and un-loading at the abattoir are the main events which caused significant increases of heart rate.

Heart rate is frequently used as a sign of an autonomic response to stress and welfare of animals during exposure to acute stressors (Fraser and Broom, 1990). According to the report of Rubio et al., (1989) heart rate is related to body weight as confirmed by investigation. In the present experiment, the heart rate at rest gave an indication for the next heart rate as it is in stressful conditions. Response during loading is greatly influenced by the animal itself, the facilities, loading ramp, experience, handling in the farm, and animal variability. Maximum heart rate was clearly observed based on the strength of stressors.

A variety of factors, such as prior experience, genetics, age, sex, or even physiological condition, shape the nature of an animal's response to a stressor (Moberg, 1987;Geers et al., 1995). Mathematical modeling is a powerful approach to understand the complexity of biological systems. Time-variant multivariate techniques are able to perform autoregressive spectral estimation and decomposition in components on a beat-to-beat basis and for more than one signal at a time. The heart rate peak and the percentage of the peak heart rate in relation to the maximum heart rate predicted for age during the cardiopulmonary exercise test were the same between the optimized and non-optimized low-sensibility (Carvalho et al., 2009). Heart rate and nonlinear deterministic shares were higher, and heart rate variability in the time and frequency domains was lower, later in the day, in cows with higher body weight (Hagen K. et al., 2005).

The above literature review contributed to the knowledge on the variability of heart rate. As heart rate is an important parameter to be considered in animal transport detail study is necessary particularly on the effect of loading during transport has not been studied enough or in detail and questions without answer.

2. Objective

Even though important research has been reported related to animal transport and welfare, still remains many questions to be addressed, particularly on the effect of transport time, vibration and climatic conditions. The aim of this thesis was to assess a system for animal transport processes such as loading, unloading, transport time and vibration that has relation with stress which needs improvement and continuous investigation.

The specific objectives were to:

- a. investigate the dynamic performances of heart rate responses of heifers and cows during loading for transport, and to describe the heart rate time series using a dynamic simulation model.
- b. determine the vibration levels and frequencies of a typical vehicle used for transporting cattle; and to study the vibration level transferred from vehicle to animals during transport.
- c. investigate the effect of transport times of up to 12 hours on the welfare of pigs when transported under conventional condition in relation to thermal stress, stress hormones, behavioural alteration and pH values.

Structure of the work

The structure of this work is schematically shown in Figure 3. In the thesis, Paper I, II and III mainly focus on animal transport by vehicle. The objective is to study the effect of climatic condition, loading, transport, unloading and vibration on animal's welfare during transport in Sweden. The stress response parameters that were considered for each paper are reported in Table 1.



Figure 3. Structure of the work

3. Methodology

The main methodology includes field measurement, data analysis and simulation

In paper I, a field experiment was carried out using 18 cattle (11 heifers and 7 cows). The cattle were transported from two farms to the abattoir. The instrument package manufactured by Polar Electro Oy Finland (Gebresenbet et al., 2010) was used for heart rate measurement.

The two parameters describing the rising rate and recovery rate of heart were determined with PowerOpt. The model simulates the heart rate of individual animal at any time within the time window of loading activity.

In paper II, during field experiments five cows were used for each experiment and a typical cattle truck was used for transport. The vehicle had a single deck, an air suspension system and was fitted with two pens separated by a steel gate. All analyses were performed using Matlab software (version R2009b). Statistical data analysis has also been conducted with SAS software, using GLM and ANOVA procedures.

In paper III, 2753 pigs were transported from various farms to the abattoir and out of these 216 were transported in the observation box and behavioural study was made on them. However, 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 transported. The age of the pigs was six months and their average weight was 100 kg.

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 using General Linear Model (GLM), Multivariate analysis of variance (MANOVA) and clustering (dendrogram).

3.1. Parameters

Table 1. Stress induced and response parameters considered in each	
research papers (I-III)	

Stress parameters considered	Paper-I	Paper-II	Paper-III
Stress induced parameters	Loading	Road conditions Speed Vibration Standing orientation	Transport time Temperature Relative humidity
Stress response parameters	Heart rate	Physiological stress Emotional stress Behavioural change	Stress hormones pH Behavioural change

3.2. Heart rate measurement during loading

The cattle were transported from two farms to the abattoir and animals were Swedish red breed. Measurement of heart rate started before loading to obtain data of heart rate at resting conditions and continued until the end of loading activities. Polar transmitter with elastic belt and polar heart rate monitor devices were used to measure the dynamic responses of heart rate of cattle during loading for transport. The heart rate recording process were started by mounting the sensor on the chest cavity of the animal as shown in Figure 4 and heart rate data was recorded and transferred to computer for analysis. The heart rate data were recorded on a beat-to-beat basis.



Figure 4. Heart rate sensors and receiver mounted on cow

3.3. Modeling of heart rate

The dynamic response of heart-rate of cattle during loading was modeled and studied. To describe the pattern of heart rate response of cattle, an exponential function was used. Powersim simulation software which utilizes the system dynamics method was used to model heart-rate of cattle. Figure 5 presents the flow chart used in developing the simulation model of heart rate of cattle during loading for transport.



Figure 5. Flow chart of algorithm used to model cattle heart rate.

The mathematical expression used to describe the pattern of animals' heart rate signals during loading was:

$$HR(t) = - \begin{cases} HR_{rest} + A_1 e^{r_1(t-T_1)} \dots t \leq T_1 \\ \dots \dots t \leq T_1 \\ HR_{rec} + A_2 e^{-r_2(t-T_1)} \dots t \geq T_1 \end{cases}$$
(1)

Where HR(t) is a dependent variable, heart rate at time t (time t is independent variable)



Figure 6. Heart rate responses during loading for transport

Powersim software was used to build the simulation model. Measured parameters such as heart rate related parameters (HR_{rest} , HR_{max} , HR_{rest} , T_1 , T_2) were obtained from recorded data. Parameters such as r_1 and r_2 were determined using PowerOpt, a software package that works interactively with Powersim software. During model verification and validation, a least square technique was used to determine the values of parameters. The performance of the model was quantified by calculating the coefficient of determination, R^2 . The value of R^2 was determined for heart rate data of each animal involved in the experiment.

3.4. Vibration measurement and analysis

In the current study dairy cows from 20 farms (95 in total, 62 Swedish Red and White and 33 Holstein) were used in field experiments. A typical cattle truck (Volvo FM 12 4X2 type) was used for transport. The vehicle had a single deck and fitted two pens separated by a steel gate. The vehicle which was adjusted with air suspension, was driven at 30, 50, 70 or 90 km/h on three road types RT1, RT2 and RT3 (See Figure 7)



Figure 7. Road types used during experiment

Vibration levels were measured at two positions on the vehicle, using Acc1 and Acc2 vibration sensors mounted on the chassis and floor as shown (Figure 8 (a)). These sensors were connected to the computer by a cable. Vibration levels on the animals were measured in three directions using tri-axial accelerometers (Acc3) using sensors shown (Figure 8(b)). The logger containing sensors was mounted with tape on a girth belt around each animal's chest. And on each trip, measurements were made on five animals simultaneously. 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.



Figure 8. Measurement sensors mounted on vehicle and animal to measure vibration levels and loggers used to record vibration levels (Gebresenbet, 1997)

The parameters, root mean square (r.m.s.), crest factor (CF), vibration dose value (VDV), estimated vibration dose value (eVDV), transmissibility and power spectrum density (PSD) were measured. All analyses were performed using Matlab software (version R2009b). Statistical data analysis has also been conducted with SAS software, using GLM and ANOVA procedures.

Acceleration values were measured and used to estimate transmissibility from chassis surface to floor and from floor to animal, while r.m.s. and VDV were used similarly to evaluate the level of transmissibility. According to European Council Directive 2002/44/EC (EC, 2002) a daily limit value for exposure to vibrations is specified $1.15 m/s^2$ and a daily exposure action value of $0.5 m/s^2$ for 8 *h* reference period. Based on these values, vibration exposure was determined for 8 hours transport time using frequency-weighted *root mean square* from measured data by using the formulas:

• In vertical direction, $a_x(8) = a_{wx} \sqrt{\frac{T_{exp}}{T_0}}$

• In horizontal direction,
$$a_y(8) = 1.4 a_{wy} \sqrt{\frac{T_{exp}}{T_0}}$$

• In lateral direction,
$$a_z(8) = 1.4 \ a_{wz} \sqrt{\frac{T_{exp}}{T_0}}$$

Where T_{exp} - is exposure time to vibration during transport and T_0 - is total transport time. a_{wx} , a_{wy} and a_{wz} are frequency weighted r.m.s acceleration in vertical, horizontal and lateral directions whereas 1.4 is multiplying factor only for lateral and horizontal axes.

Analysis of Vibration parameters

★ The crest factor is defined as the modulus of the ratio of the maximum instantaneous peak value of the frequency weighted acceleration signal, $\max(a_w(t))$, to its frequency-weighted root mean square acceleration value (a_w) . The crest factor can be calculated according to (Griffin, 1990) as:

$$CF = \frac{\max(a_w(t))}{a_w}$$
 Where CF is the crest factor

Acceleration on the chassis was used as input with respect to the floor. Floor acceleration was used as input with respect to cattle acceleration. Transmissibility is used to describe the effectiveness of a vibration isolation system, expressed as the ratio of input to output (Griffin, 1990).

$$Transmissibility = \frac{a_i floor}{a_i chassis}$$
$$Transmissibility = \frac{a_i animal}{a_i floor}$$

- The parameter estimated vibration dose value (eVDV) is calculated to assess the net vibration absorbed by the body during period of exposure whereas VDV, cumulative measure of vibration, and should be used to determine the total severity of vibrations.
- Vibration dose value (VDV) and estimated vibration dose value (eVDV) are determined according to Griffin (1990)

$$VDV = \left[\frac{T_s}{N}\sum a^4(i)\right]^{\frac{1}{4}}$$
 and

$$eVDV = [(1.4R)^4 T_S]^{\frac{1}{4}}$$

Where T_s is sampling period, a(i) - is acceleration data, N-is number of observations and R- is root mean square acceleration.

3.5. Transport Time

3.5.1. Blood parameters and analysis

Measurement and analysis of stress hormones were done to study the effect of transport time on animal welfare. Cortisol, creatine kinase, lactate and glucose were parameters considered and blood samples were taken before transport at the farm and after unloading at the abattoir. Blood samples were taken from a total of 90 pigs for the determination of concentration levels of stress hormones. Every animal was bled twice, at the farm before the start of transportation and immediately after transportation and subsequent stunning at the abattoir. Blood samples were taken from jugular vein. The cortisol values in transported and control pigs were measured using radioimmunoassay Coat-A-Count cortisol kits. Serum glucose and Creatine kinase was analysed using an automatic Konelab analyser and lactate levels were measured using a GM7 Analox analyser. Blood samples were taken also from pigs that were not transported. The values obtained from the control pigs were used when performing statistical analysis of the samples gathered during the field experiment.

3.5.2. pH measurement

During the study meat samples were taken from the longissimus dorsi (LD) for pH determination. The carcases were chilled for 24 h at $+4^{\circ}$ C. During that time, the decreased temperature and pH were measured in longissimus dorsi between the 12th and 13th rib immediately after slaughter, and at 0, 5, 18 h and 24 h post-mortem.

3.5.3. Behavioural parameters

Pigs behaviours were continuously observed and documented at farms (during blood sampling and loading), during transport (in the vehicle) and unloading at the abattoir by visual observation, portable and fixed video cameras. To evaluate behavioural alterations in response to handling and transport activities, the most common observed behaviours were selected and definitions were given for all selected behaviours. For determination of frequency, occurrence of events and total number of animals in the observation box were used. Therefore, the final quantified behaviour was expressed as the product of frequency and duration of events.

$$Frequency = \frac{A}{B}$$

 $Behaviour = Frequency \times t$

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

4. Results

4.1. Dynamic response of cattle heart rate during loading for transport

Heart rate is a stress response parameter that describes animal response to physical and psychological stress. The heart rate variability of cows and heifers was observed as it increases exponentially from resting point to peak value and decreases exponentially from peak point slowly up to the recovery level. The difference between rising and recovery amplitude increases as rising amplitude increases and the increase in the difference between the rising and recovery amplitude showed that animal stayed under stress condition, heart-rate did not fully recovered to its resting level (See Figure 9 (b)). But as the smaller the difference between the rising and recovery amplitude the less stress or the more animal adapted to the new environment. The relationship between rising rate (r_1) and period (T_1) , recovery rate (r_2) and period (T_2) were investigated and evidenced. The values of r_1 and r_2 decreased as T_1 and T_2 increased. In general it was noticed that the mean value of rising rate was nearly twice the recovery rate value for both animals, this showed that the heart-rate rises more rapidly and recovered slowly.

The recorded and simulated curve of two animals showed the heart rate pattern during loading. Figure 9 (a) curve shows when the heart rate is fully recovered, whereas Figure 9 (b) curve indicates partially recovered heart rate. The model could simulate the pattern of heart rate response. During simulation it was noticed that the simulated values of HR_{rest} , HR_{max} , and HR_{rec} , were almost the same to the recorded values for each subject.



Figure 9. Simulated and recorded Curves of heart rate pattern during loading (a) curve showed when the heart rate fully recovered (b) curve indicated partially recovered heart rate

The result of current study showed that the heart-rate of cows and heifers increased exponentially from resting level to peak value and the values of $HR_{rest.}$, HR_{max} and HR_{rec} were higher for heifers than cows. The mean heart rate at resting condition, peak, and after recovery was $80 \pm 6 \ bpm$, 136+ 35 bpm and 91+ 19 bpm for heifers and 47+4 bpm, 102+ 27 bpm, and $55\pm 12 \ bpm$ for cows, respectively. In general, during stress, the heart rate was raised exponentially from its mean resting value to peak value about 1.9 times the value at resting level. And during the recovery period the heart rate declined and maintained steady state at HR_{rec} value, about 1.15 times the resting value, on average. The simulated data was directly correlated with the recorded data and the coefficient of determination noted were $R^2 = 0.89 \pm 0.06$. The pattern of heart-rate response and the mean values of R^2 was the same for both cows and heifers, there is no significant differences. Considering all together, for the heifers and cows, the amplitude A_1 was 55 ± 27 bpm and A_2 was 46 ± 20 bpm. A₃ indicates the difference between the rising and falling amplitudes that its value varied from 0 - 42 bpm. The relationship between A_1 and A_3 was noticed that A_3 increases with an increase of A_1 (see Figure 10(a)). High A_3 value suggests that the animal was under more stress condition and heart rate did not fully recovered to the resting level (see Figure 9(b)).



Figure 10. Relation between parameters (a) The relation between A_3 and A_1 (b) The relation between r_1 and T_1 (c) The relation between r_2 and T_2

4.2. Vibration levels and frequencies on vehicle and animals during transport

In the current study the result showed that the transmissibility of vibration level from the chassis to the floor damped as the vibration level from the vehicle floor to the animal was amplified. The upper part of the animals' body was swaying in relation to the footing and therefore vibration level on upper part of the body of animal could be higher than on the floor. Detailed results are presented in Table 2. The highest level of vibration values recorded on animals on gravel roads was in driving speeds of 50 km/h and 70 km/h in the driving direction.

	Floor/ Chassis				Cattle/ Vehicle floor			
Parameter	Vertical Horizontal		Lateral	Vertical	Horizontal	Lateral		
а	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.ms.	0.48 ± 0.15	0.51 ± 0.13	0.66±0.12	0.95 ± 0.18	1.1 ±0.43	1.43 ±0.32		

Table 2. Transmissibility of acceleration, VDV and r.m.s. from chassis to floor and from floor to cattle

Vibrations in the horizontal and lateral directions were lower on animals positioned perpendicular to the direction of travel than on those facing forward. Both road conditions (P ≤ 0.0002) and standing orientation (P ≤ 0.002) have a significant effect on vibration levels.

The results of VDV, eVDV, *r.m.s.* and CF during transport on tarmac roads noted. In the three orthogonal axes, the range of values of VDV (4.13 ± 0.76 to $8.35 \pm 2.56 \frac{m}{s^{1.75}}$), eVDV (4.25 ± 0.61 to $7.96 \pm 2.36 \frac{m}{s^{1.75}}$) and *r.m.s.* (0.81 ± 0.12 to $1.52 \pm 0.45 \frac{m}{s^2}$) was higher on the chassis than on the vehicles floor and cattle. Furthermore, VDV, eVDV and *r.m.s.* were higher on cattle than on the floor along the horizontal and lateral directions, but lower in the vertical direction. The highest CF on the floor was 6.6 and on animals 5.6 (see Table 3).

Parameters	Vertical	Horizontal	Lateral
Chassis CF	5.07±1.1	5.68±0.9	6.55 <u>+</u> 0.9
Floor CF	5.28±1.7	6.64±2.2	3.7±0.7
Animal CF	3.97 <u>±</u> 1.6	5.6±5.1	2.86±0.9

Table: 3. Measured Crest factor on (chassis, vehicle floor and animal) in three orthogonal axes

The smallest crest factor occurred on cattle than vehicle and it is within the approximate range for road vehicles suggested by Griffin (1990), which is

between 3 and 9. Three main resonance frequencies were identified for the vertical direction, at 1.3, 5.1, and 12.6 Hz, and a secondary peak at about 23 Hz on tarmac road with the speed of 85km/h.

4.3. Effects of transport times on welfare of pigs

4.3.1. Stress hormone parameters

The concentration of cortisol was significant (P ≤ 0.001) and elevated during short transport time and the rate of elevation decreased with an increase of transport time (see Figure 11). The rate of elevation during winter was 58.2 - 25.3 nmol L⁻¹ while during summer 59.2-31.8nmolL⁻¹.



Figure 11. Cortisol concentration during winter and summer

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



Figure 12. Glucose concentration level during winter and summer

Concentrations of lactate positively correlated with transport time, increased as transport time increased (See Figure 13). Lactate concentration varied between 4.2 and 7.3*mmol* L^{-1} . Concentration levels of lactate during winter in the blood increased from 4.7 to 6.2 *mmol* L^{-1} with an increase in transport time and positively correlated (R²=1) (P ≤0.002).



Figure 13. Lactate concentration during winter and summer

Concentrations of creatine kinase positively correlated with transport time (See Figure 14). The elevation in concentration levels of creatine kinase ranged from 0.4 to 25.4 $\mu molL^{-1}$ during winter and from 2.5 to 31 $\mu mol L^{-1}$ during summer and thus positively correlated with transport time (P<0.002). The relationship between concentration level and transport time for summer and winter seasons (R² = 0.99). The rate of increase in creatine kinase concentration from 4 h to 8 h was lower than from 8 h to 12 h transport time (See Figure 14). During the summer season and 12 h transport time, the maximum value after transport was 154 $\mu molL^{-1}$, and exceeded the reference value of 129 $\mu molL^{-1}$.



Figure 14. Creatine kinase concentration level for winter and summer increased exponentially with transport time

4.3.2. Behavioural alteration and quantification

The final quantification of behaviour was expressed as the product of frequency of events with duration of events. Lying, sitting, rooting, vocalization, restless and change of position, smelling, panting, loss of balance and fighting were significant and positively correlated with transport time ($P \le 0.009$) (See Table 4). Values for rooting and vocalization were higher during loading. Behaviours like rooting, reversal and vocalization showed that the severity of stress was higher during loading than unloading.

		Trar	nsport time (h)			
Behaviour	0-4		4 - 8		8 - 12		
	Freq	Freq x	Freq	Freq x	Freq	Freq x	
		time		time		time	
Ft	1.12	0.17	0.3	0.18	0.2	0.1	
Jn	0.02	0.01	0.01	0.03	0.02	0.01	
Ln	0.02	0.14	0.05	0.19	0.07	0.13	
Ls	0.27	0.32	0.32	0.35	0.27	0.31	
Ly	1.8	33.13	2.10	403.80	4.43	614.5	
Pt	0	0	0.2	0.48	0.3	7 0.43	
Sm	0.86	2.51	0.46	1.6	0.06	1.19	
St	0.6	2.13	0.9	3.45	0.8	3.34	
Rc	1.2	0.24	0.4	0.29	0.07	0.15	
Rt	1.23	7.40	0.77	5.25	0.52	3.02	
Va						=	
vC	2.2	3.81	1.8	2.73	1.1	2.52	

Table: 4. Observed behaviours within 4 h intervals of the 12 h transport time

Lying behaviour in relation to transport time showed interesting results. During all experiments, about 50% of pigs were lying about 2 h after the vehicle started moving then about 80% of pigs were lying after 6 h transport time, and the behaviour increased in similar way until 12 h (See Figure 15).



Figure 15. Proportion of pigs lying in percentage depending on transport time, described with 4^{th} order polynomial function, with correlation coefficient, $R^2 = 0.95$.

4.3.3. pH value

In current study the lowest 5.5 and highest 6.1 pH values were at 4 h and 12 h transport time respectively. Highest pH_{24} of 5.99 ± 0.29 occurred during summer at 12 h transport time (See Figure 16 and Table 5). The measured pH_{24} values in carcases showed positively correlated with transport time during summer. The pH_{24} value during summer season for 12 h transport time exceeded 5.8, and the elevation of pH_{24} might be attributable to climatic conditions in the vehicle. The pH_{24} values during summer correlated with transport time ($R^2 = 0.69$) (see Figure 16). During summer loading caused thermal stress and inadequate ventilation after washing was the main cause for high initial relative humidity.



Figure 16. Effect of transport time on pH_{24} value in pig carcases during summer, with correlation coefficient, $R^2 = 0.69$.

Season	Transport Time (h)	pH ₀	pH ₅	pH ₁₈	pH ₂₄
Summer	4	6.54±0.32	6.15±.25	5.67±.21	5.65±.17
	8	6.42 ± 0.31	6.23±.23	5.69±.23	5.79±.18
	12	6.29±.28	6.10±.19	5.59±.19	5.99±.29
Winter	4	6.50±.08	6.49±.13	5.46±.12	5.58±.14
	8	6.50±.17	6.37±.22	5.41±.21	5.48±.12
	12	6.50±.21	6.23±.23	5.53±.19	5.50±.22

Table: 5. Summary of pH values for different seasons and transport times.

A summary of the mean pH values are presented in Table 5. The highest pH_{24} values for winter were 5.58±0.14. Table 5 shows seasonal effect on pH values at pH_0 , pH_5 , pH_{18} and final pH 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. The ultimate pH values for the 4, 8 and 12 h of transport times are showed in Table 5.

4.3.4. Temperature and relative humidity

Measurement of temperature and relative humidity were performed simultaneously and continuously throughout transport time and the temperature-humidity index (THI) was 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.

in winter and summer									
4 (h)			8 (h)			12(h)			
Seasons	Temp (°C)	RH (%)	THI (%)	Temp (°C)	RH (%)	THI (%)	Temp (°C)	RH (%)	THI (%)
Winter	4.4	88.87	41.03	3.72	90.9	39.67	4.1	72.9	42.17
Summer	17.13	94.19	62.68	15.46	89.72	59.72	18.98	94.6	65.92

Table: 6. Summary of mean values of temperature, relative humidity and THI in winter and summer

The THI mean values of both seasons and three transport times (4, 8 and 12 h) were below the threshold value of 74 and the maximum mean value was 65.92 (See Table 6). Regarding the relationship between THI and transport time, seasonal variation had more effect than transport time.

The thermal humidity index used to describe categories of heat stress associated with hot weather conditions. Certain THI values during summer were above the normal (\geq 74) level during loading. The overall THI mean values of 4, 8 and 12 h transport time for two seasons remained below the threshold (\leq 74) which is considered normal (See Table 9). THI within 4 h intervals (0-4, 4-8 and 8-12 h) of 4, 8 and12 h transport time were investigated by considering the maximum, minimum, mean and standard deviations of values of each interval. Based on the recommended thresholds values the peak thermo-humidity index (THI) value was noted to be 79.24 during the 12 h transport time, i.e. above the threshold value of 74.

5. Discussions

Assessment of stress inducing factors should contain both behavioural and physiological measures. Behavioural indicators of discomfort are attempt to escape, vocalization, kicking, or struggling (Grandin, 1997). According to Grandin (1997), the common physiological measures of stress are cortisol, beta endorphin, and heart rate. During transportation animals are subjected to stress inducing factors like vibration, noise and environmental variations in the containers and these factors were considered as acute stressor causing physiological and behavioural stress (Stephanes and Rader, 1990).

5.1. Modeling of heart rate during loading for transport

Loading is one of the most stressful components of transportation for animals (Trunkfield and Broom, 1990; Waran and Cuddeford, 1995) and heart rate in animals tended to peak during loading (Stewart et al., 2003; Stirling et al., 2008). Maria et al. (2003) reported welfare using the scoring system to evaluate the stress on cattle with special emphasis on loading and unloading.

In the current study the simulation model developed described well the dynamic heart rate response of cows and heifers during loading for transport. The mean coefficient of determination, R^2 was 0.89 ± 0.06 . The heart rate rises exponentially from its mean resting value to a peak value about 1.9 times the value at resting level and then declined during a recovery period about 1.15 times the value at resting level. The amplitude of the rising part of heart rate response was not significantly different for heifers and cows. Similarly the amplitude of the decaying part of heart rate response for heifers and cows was almost the same on average. In all cases it was observed that the heart rate value did not stay at peak value, once the peak value was attained it started decaying during the recovery period.

5.2. Effect of vibration levels and resonance frequencies during transport

During transport, particularly on rough roads, transmission of vehicle floor vibration to animals can be significant and can create uncomfortable conditions by displacing the center of gravity of the animal, resulting in body disturbance, poor welfare and ultimately impaired meat quality (Randall, 1992). Road conditions (roughness, undulation and road curvature), poor driving performance (variations in speed and vibration) and poor suspension system

are the main factors causing vibration and loss of balance during transport (Gebresenbet, 2003). The vibrations can be attributed to the structure of the vehicle, road surface, vehicle speed and driving techniques, with levels greater than 2.0 m/ s^2 reported to be extremely uncomfortable to humans (Randall, 1992). However, animals in vehicles are usually exposed to higher vibration levels during transport than the driver, whose seat is designed to reduce the vibrations from the road.

In the study of transmission of vibration and resonance frequency during transport, particularly on rough-surfaced roads, the transmission of the vehicle's floor vibration to the animals can be significant (Randall, 1992; Randall et al., 1995). Vibration transmits from vehicles chassis to the floor of the vehicle and then transmits to the loaded animal. And during transport by vehicle floor vibration affects the comfort of the animal. In this study the highest vibration level observed on animals was $2.27 \pm 0.33 \ m/s^2$ when driving on gravel roads at 70 km/h. Vibrations in the horizontal and lateral directions were lower on animals positioned perpendicular to the direction of travel than those facing forward. Both road conditions (P ≤ 0.0002) and standing orientation (P ≤ 0.002) have a significant effect on vibration levels. Three main resonance frequencies were identified for the vertical direction, at 1.3, 5.1, 12.6 Hz, and at about 23 Hz. The vibration exposure values of 8 h transport period for the vertical, horizontal and lateral directions were 0.61 \pm 0.12, 0.92 \pm 0.35, and 1 \pm 0.21 m/s^2 , respectively.

5.3. Transport time and animal welfare

During handling and transport, animals were subjected to many different potential stressors like heat, cold, poor air quality that affect the welfare and health of animals up to death (Gebresenbet 2010). Animal welfare is usually defined in reference to the adaptation ability of animals to cope with changes in environmental conditions. When the pressure exerted by the environment on animal reaches a certain level, new defence mechanisms can be initiated in response to the new conditions and this response mechanism referred to stress responses (Gebresenbet et.al, 2010).

During transport cortisol, glucose, lactate and creatine kinase are relevant parameters to describe the stress levels imposed on animals. In this study cortisol concentration level was highest at 4 h transport time and generally inversely proportional to transport time. Glucose concentrations were highest at 8h and lowest at 12h transport time for winter and summer experiments, respectively. Concentration level of lactate increased with an increase in transport time during winter.

Concentration level of creatine kinase elevated exponentially ($R^2 = 0.99$) as transport time increased from 4 to 8 and to 12 h. 154 μ mol l^{-1} was the maximum value noted at 12 h transport time during summer experiments and the control values varied between 115 – 129 μ mol l^{-1} . Behavioural alterations, particularly lying, sitting, rooting, vocalisation, smelling

restlessness and change of position and panting were correlated with transport time.

5.4. Synthesis

In this thesis, a comprehensive study has been made covering the conditions of transport and its effect on animal welfare. The most important stress inducing factors considered were loading and unloading; vibration, transport time and climatic conditions in the vehicle. The main stress response parameters were blood hormones, heart rate and behavioural alteration. The combination of these parameters was necessary to describe the welfare status of animals as the welfare of animal is the reflection of physiological and behavioural condition and general well-being. An animal in a poor state of welfare suffer from discomfort, distress, pain, injury, malnutrition and thirst which may compromise its ability to cope with its environment. As described earlier, elsewhere in the thesis; the whole thesis is structured in to three papers (see Figure 3) to provide answers to the main questions of what the major parameters consider to improve animal welfare. Vibration and transport time are very much linked to each other. To subject animals to vibration for a long time could cause trauma on animal and that is why it was important to study these parameters.

The rising and falling of heart rate during stress is not always the same it depends on the strength of stressor. The vibration dose value estimated vibration dose value and r.m.s. were higher on animal than on the floor of the vehicle along the lateral direction and lower on animal than on the floor of the vehicle along the vertical direction. The highest vibration levels were observed on animals on gravel roads in different driving speeds and direction. Behaviours like rooting, reversal and vocalization showed that the severity of stress and higher during loading.

Description and statistical analysis, and modeling were the methodologies employed. Generally, it is not possible to eliminate the stress all in all. But major stressor could be reduced by improving facilities and method of handling.

6. Conclusions

Based on transport conditions, vibration levels, animal behaviour, stress hormones and pH_{24} value, it can be concluded that transport and its process has an adverse effect on animal welfare. Transport time had significant effects on pigs' welfare as showed by blood parameters and behavioural parameters.

Modeling dynamic responses of heart rate of heifers and cows during loading showed that the mean value of rising rate was nearly twice the recovery rate value for both animals (heifers and cows). The heart rate of cows and heifers increased exponentially from resting level up to peak value and the values of $HR_{rest,}$, HR_{max} and HR_{rec} of heifers higher than cows. The mean heart rate at resting condition, peak, and after recovery was $80\pm 6bpm$, $136\pm 35 bpm$ and $91\pm 19 bpm$ for heifers and $47\pm 4bpm$, $102\pm 27bpm$, and $55\pm 12 bpm$ for cows, respectively.

The highest vibration values recorded on animals on gravel roads in driving speeds of 50 km/h and 70km/h in the driving direction.Vibrations in the horizontal and lateral directions were lower on animals positioned perpendicular to the direction of travel than on those facing forward. The transmissibility of vibration level from the chassis to the floor damped whereas the vibration level from the vehicle floor to animal is amplified. The vibration dose value estimated vibration dose value and r.m.s. were higher on animal than on the floor of the vehicle along the lateral direction and lower on animal than on the floor of the vehicle along the vertical direction. Both road conditions ($P \le 0.0002$) and standing orientation ($P \le 0.002$) had a significant effect on vibration levels. Three main resonance frequencies were identified for the vertical direction, at 1.3, 5.1, 12.6 Hz, and at about 23 Hz. The vibration exposure values of 8 h transport period for the vertical, horizontal and lateral directions were 0.61 \pm 0.12, 0.92 \pm 0.35, and 1 \pm 0.21 m/s^2 , respectively. These exceed the EU daily exposure action of $0.5 m/s^2$, but lower than the daily exposure limit of $1.15 m/s^2$.

Regarding the effect of transport time on the welfare of pigs, cortisol concentration was elevated during short transport time and significant ($P \le 0.001$). However the concentration level of cortisol was inversely proportional to transport time. Glucose concentration increased from 4 h to 8 h and thereafter decreased slightly ($P \le 0.01$). Lactate concentrations (winter) positively correlated ($P \le 0.002$; $R^2 = 1$) with transport time. Creatine kinase concentration was positively correlated with transport time ($P \le 0.01$; $R^2 = 0.99$).

Behaviours alteration lying, sitting, rooting, vocalization and smelling were scored high values and positively correlated with transport time. Behaviours like rooting, reversal and vocalization showed that the severity of stress was higher during loading than unloading. The measured pH_{24} values in carcases showed positively correlated with transport time during summer. The pH_{24} value during summer season for 12 h transport time exceeded 5.8, and the elevation of pH_{24} might be attributable to climatic conditions in the vehicle, where the peak thermo-humidity index (THI) value was also noted to be 79.24 during the 12 h transport time, i.e. above the threshold value of 74. During summer loading caused thermal stress. The overall conclusion from the study, based on climatic conditions, animal behaviour, stress hormones, and final pH values in carcases, was that an increase in transport time from 4 h to 8 h had a higher effect than an increase from 8 h to 12 h on the welfare and subsequent meat quality.

Future Research

In this study, many issues were raised and investigated but some remain which need further studies. Some of the important remaining issues are presented below:

- Vibration and its consequence of motion during transport affect the welfare of animals and it is harmful for animals that are exposed to it. In the current study, the vibration levels on vehicle chassis, floor and animal were investigated. The consequences of vibrations in relation to animals heart rate were not investigated which requires further research to know how it affects animals' heart rate during transport.
- During transportation of pigs, behavioural occurrences were highly and repeatedly observed but the reason for these behavioural changes needs further study in relation to the effect of vibration, to determine and identify its impact on heart rate of pigs. Vehicle motion and vibration could have effects on pigs, in creating motion sickness, unhealthy, discomfort and postural instability. These adverse effects need further investigation.

References

- Broom D. M., (2006). Behaviour and welfare in relation to pathology, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK, Applied Animal Behaviour Science 97, 73–83
- Broom Donald M. (2000). Cause of poor welfare and welfare assessment during handling and transport. Centre of animal welfare and Anthrozoology, Department of veterinary medicine, University of Cambridge, Cambridge UK
- Buzdugan, G., Miha` ilescu, E., & Rades, M. (1986). Vibration measurement, Bucharest: Martinus Nijhoff Publishers.
- Buckham Sporer K R, Weber P S D, Burton J L,Earley B and Crowe M. A. (2008). Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. J. Anim Sci. 2008
- Fraser, A. F., and D. M. Broom. (1990). Farm Animal Behaviour and Welfare (3rd Ed.). Bailliere Tindall, London.
- Gebresenbet, G., Kaumbutho, P.G., Simpkin, P., (2006). Work Performance and Physiological Response of Camels: Research Project Report. Swedish University of Agricultural Sciences, Uppsala.
- Gebresenbet and Eriksson (1998). Effects of transport and handling on animal welfare, meat quality and environment with special emphasis on tied cows. Swedish university of Agricultural Sciences, Project Report 33:1998.
- Gebresenbet, G. (2003). Current research on animal transport: Evaluation and recommendations. Sentient beings or insensitive goods. Swedish Government Official Reports. SOU. 267e305.
- Gebresenbet G, Aradom S, Bulitta FS, Adam M (2010). Effect of Transport Time of up to 12 Hours on Animal Welfare. Project Report, Swedish University of Agricultural Sciences, Uppsala.
- Gebresenbet, Girma and Wikner, Isabelle and Ladberg, Eva and Holm, Patrik and Nilsson, Christer and Svensson, Lars (2005). Effect of transport times on cattle welfare and meat quality. Department of Biometry and Engineering Swedish university of Agricultural Sciences. SLU, Sweden.
- G. Gebresenbet and K. Sälvik (2006). Livestock production and society, Animal welfare aspects, the technical challenge during housing, transport and slaughter: Dairy cows. Chap – XVIII (page 257-

268). Swedish university of agricultural sciences, Department of Biometry and Engineering, Uppsala, Sweden,

- Geers, R., G. Janssen, H. Ville', E. Bleus, H. Gerard, S. Janssen, and J. Jourquin. (1995). Effect of human contact on heart rate of pigs. Anim. Welf. 4:351-359.
- Grandin T., (1997). Assessment of Stress during Handling and Transport, Journal of Animal Science, volume 75: 249-257
- Grandin T., (1978). Transport from the animal's point of view. American Soc. of Agric. Engineers. Paper No. 78–6013.
- Gregory N. G., (2008). Animal welfare at markets and during transport and slaughter. Meat science 80, 2-11. BBSRC and Royal Veterinary College, Hatfield, AL97TA, UK
- Griffin, M. J. (1990). Handbook of human vibration. Cornwall: T.J Press. ISO (1997). International organisation for standardardization. Guide for the evaluation of human exposure to whole body vibration. ISO. 2631.
- Graham B. Scott (1994). Effects of short-term whole body vibration on animals with particular reference to poultry Worlds poultry science journal, 50 : pp 25-38, UK.
- Kenny, F. J.; Tarrant, P. V. (1987c). The behaviour of young Frisian bulls during social regrouping at an abattoir. Influence of an overhead electrified wire grid. Applied Animal Behaviour Science 18, 233-335
- Knowles, T. G.; Warriss, P. D.; Brown, S. N.; Edwards, J. E. (1999). Effects on cattle transportation by road for up to 31 hours. *Veterinary Record* 145, 575-582
- K. Hagena, J. Langbeinb, Schmieda, Lexera, Waiblingera (2005). Heart rate variability in dairy cows—influences of breed and milking system. University of Veterinary Medicine Vienna, Department of Veterinary Public Health and Food Science, Institute of Animal Husbandry and Animal Welfare, Veterina rplatz 1, A-1210 Vienna, Austria, Physiology & Behavior 85 (2005) 195 – 204
- Lambooij E., Putten G. van, Van Putten G. and Grandin T. (1993). Transport of pigs In: Livestock Handling and Transport. 213-231.
- María, G., Villaroel, M., Gebresenbet, G. (2003). Scoring system for evaluating the stress to cattle of commercial loading and unloading. The Veterinary Record, volume 154, issue 26, 818-821
- Moberg, G. P. (1987). A model for assessing the impact of behavioral stress on domestic animals. J. Anim. Sci. 65:1228-1235
- Murata, H. and H. Hirose (1991). Suppression of bovine lymphocyte and macrophage functions by sera from road transported calves. *Brit. Vet. J.* <u>147</u>, *S.* 455-462

- Perremans, S., J. M. Randall, M. A. Stiles, L. Allegaert, J. Mast, B. Goddeeris, and R. Geers. (1996). Quantification of piglet response to vibration during vertical motion. Proc. 14th IPVS Congr., Bologna, Italy, July 7-10. p 741.
- Randall J.M., (1992). Human subjective response to lorry vibration: Implications for farm animal transport. J. Agricult. Eng. Res. 52: 295-307.
- Randall J.M., Duggan J.A., Alami M.A., (1995). Influence of motion and vibration on animals. *Fleischwirtsch*. 75: 158-160.
- Rubio, D., R. Santisteban, F. M. Castejon, and P. Tovar. (1989). Influence of age on the electrical auricular systole in swine. J. Vet. Med. A 36:64-70.
- Stephanes and Rader., (1990). The effects of restraint, handling, simulated and real transport in the pig (with reference to man and other species). Applied Animal behaviourScience, Volume28, issue1, page41-55.
 Stewart (2003), Stewart M, Foster TM, Waas JR (2003) The effects of air transport on the behaviour and heart rate of horses. Applied Animal Behaviour Science 80:143-160.
- Stirling JR, Zakynthinaki M, Refoyo I, Sampedro J (2008). A model of heart rate kinetics in response to exercise. Journal of Nonlinear Mathematical Physics 3: 426-436.
- Tadich N., Gallo C., Bustamante H., Schwerter M., Van Schaik, G., (2005). Effects of transport and lairage time on some blood constituents of Friesian-cross steers in Chile. Livest. Prod. Sci. 93:223-233.
- Trunkfield HR, Broom DM (1990). The welfare of calves during handling and transport. Applied Animal Behaviour Science 28:135-152.
- Vitor Oliveira Carvalho, Rodrigo Xavier Rodrigues Alves, Edimar Alcides Bocchi, Guilherme Veiga Guimarães (2009). Heart rate dynamic during an exercise test in heart failure patients with different sensibilities of the therapy Heart rate dynamic during exercise test . International Journal of Cardiology
- Waran NK, Cuddeford D (1995). Effects of loading and transport on the heart rate and behavior of horses. Applied Animal Behaviour Science 43:71-81.
- Winckler C, Capdeville J., Gebresenbet G, Hörning B., Roiha U., Tosi M., Waiblinger S., (2003). Selection of parameters for on-farm welfare assessment protocols in cattle and buffalo. Animal Welfare 12, 619-624
- Yu J., Tang S., Bao E., Zhang M., Hao Q., Yue Z., (2009). The effect of transportation on the expression of heat shock protiens and meat quality of M. longissimus dorsi in pigs. Meat Science 83, 474- 478. College of veterinary Medicine, Nanjing Agricultural University, Weigang 1.Nanjing 210095, China.

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