Effects of Handling on Animals Welfare during Transport and Marketing

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Cover: Animal transport by walking/ trekking from Gudar to Finfinnee, Ethiopia.
(Photo: by FUFA SORRI BULITTA)

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Abstract

Animals can be transported either by trekking or by vehicle, during which they are subjected to different types and levels of stressor. Some key factors affecting animal welfare during handling and transport are mixing of unfamiliar animals, handling procedures, driving methods, stocking density, journey length, vehicle design, animal standing orientation, loading and unloading facilities and transport by walking. Much important research has been done on animal transport and welfare, but many questions remained to be addressed, particularly regarding the effects of transport time and length, vibration, climate conditions and handling during transport and marketing.

This thesis investigated the effects of handling on animal welfare during transport and marketing. The main methods employed were comprehensive field measurements to collect data, observations, video filming for behavioural studies, interviews with animal handlers and heart rate modelling. The results showed that during loading for transport, animal heart rate rose exponentially from its mean resting value to a peak value and declined during a recovery period. Driving speed, road conditions and the standing orientation of animals had an effect on levels of vibration. The three most common resonance frequencies identified were 1.3, 5.1 and 12.6 Hz, with a second peak at 23 Hz in the vertical direction on a tarmac road at a driving speed of 85 km/h. In pig and cattle transport, blood cortisol level was elevated during short transport time. Concentrations of lactate and Creatine kinase and animal behaviour were positively correlated with transport time.

During transport of animals by trekking from farm to feeder market and on to regional market in Ethiopia, the number of animals that died, were injured and were stolen was 7.6%, 6.9% and 2.8%, respectively.

The overall conclusion from the thesis, based on transport conditions, vibration levels, animal behaviour, stress hormones and pH_{24} values, was that handling and transport had a negative effect on animal welfare.

***Keywords*:** animal welfare, transport time, stress parameters, heart-rate, loading, behaviour, vibration

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

"Rakkoowwan namni namarraan qaqqabsiisu hundumaa yoo falmatan ykn tasgabahanii hiikoo itti Kennan bakka barbaadan ni ga'u garuu daandiin isaa dheeraa dha!" Uumaan Oromoo Waaqnis nama gargaara!!

"SALGAN CULULLEE MANNAA TOKKICHA RISAA WAYYA!!"

Mamaaksa Oromoo

FSB

Contents

List of Publications

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

I Bulitta FS, Bosona TG, Gebresenbet G. (2010).Modelling 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. (2010). 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 Pigs *Journal of Agricultural Science and Technology A 2 (2012) 544-562.*

IV Fufa Sorri Bulitta, Samuel Aradom, Girma Gebresenbet,(2015). Effect of Transport Time of up to 12 hours on welfare of Cows and Bulls. *Journal of Service Science and Management, (2015) 8, 161-182.*

V Bulitta Fufa S., Gebresenbet G., Bosona T., (2012). Animal Handling during supply for marketing and operation at abattoir in developing country: The case of Gudar market and Ambo Abattoir, Ethiopia. *Journal of Service Science and Management, Volume 5, 59-68.*

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The contribution of Fufa Sorri Bulitta in each research papers (I- V) included in this thesis was as follows:

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

II Paper II: Data analysis and writing of the paper together with other co-authors

III Paper III: Data analysis and writing of the paper together with other co -authors

IV Paper IV: Data analysis and writing of the paper together with other co-authors

V Paper V: Planned data collection method, data analysis and writing of the paper together with other Co-authors

Abbreviation

A_1 Rising amplitude

A_2 Falling Amplitude

A_3 The difference between rising and falling amplitude

acc_1, acc_2 and acc_3 Vibration sensors mounted on chassis, vehicle floor and on animals respectively

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

HR_O Heart rate in *bpm* at initial resting condition

PSE Pale, Soft, Exudative

PSD Power spectral density

r_1 Rising rate

r_2 Falling rate

r.m.s. Root mean square

RT_1, RT_2 and RT_3 Gravel or poor tarmac road, Good tarmac road and Motorway

T_1&T_2 Rising & Falling time

t Time in second

VDV Vibration Dose Value

1 Introduction

1.1. Background

Animals are transported for different reasons, for marketing, slaughter, fattening and breeding (Grandin, 1978). Transport and handling of animals are very complex activities which compromise animal welfare and meat quality. During transport, animals are exposed to different stressors such as vibration, environmental variations in the vehicle, noise, high temperature and high relative humidity (Gebresenbet & Ericsson, 1998). Animals feel pain, discomfort and suffering during poor handling and transport. The EU has developed various directives and pieces of legislation to improve animal welfare during transport (EC 1/2005) and this can act as guidance for hauliers on transporting farm animals in a safe way from farm to abattoir or other destinations. The legislation outlines the level of risk related to various aspects of animal transport, such as means of transport, transport processes and space allowances of animals ((EC 1/2005).

The welfare of animals could be improved if animal producers, handlers and transporters improved their knowledge of how animals are affected by 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, 2000). In general, ensuring animal welfare is a human responsibility which includes consideration of all aspects of animal welfare, including proper housing, management, nutrition, disease prevention and treatment, responsible care and humane handling. Transport is a major stressor that has effects on the health, well-being and performance of animals.

Poor transportation systems and processes in particular have serious effects on the welfare of animals. The EU has rules that govern animal welfare during transport which aim to eliminate technical barriers to transport and trade of farm animals and to allow market organisations to operate smoothly, while ensuring a satisfactory level of protection for the animals (EC 1/ 2005). The stress caused by handling and transport is largely induced in animals in different ways, as illustrated in Figure 1. The diagram also shows some of the psychological and physiological disturbances that stressful conditions can cause under different transport conditions. Any stress is generally the result of a stressor, whether of external or internal origin. The level of an animal's response to the stress imposed on it depends on that animal's level of perception.

External factors may stimulate animals in a way that the animals perceive as positive, and this result in good welfare and better meat quality. However, if animals perceive the stimulation as negative; it leads 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 physiological disturbances. During transport, animals are subjected to vibration, which leads to loss of balance, travel sickness, food and water deprivation, fear, fatigue etc. Hence, determination of vibration level is required to improve the design of vehicles. Length of journey time can also affect animal health and welfare, but there is no adequate scientific work to date to determine the limits of transport time.

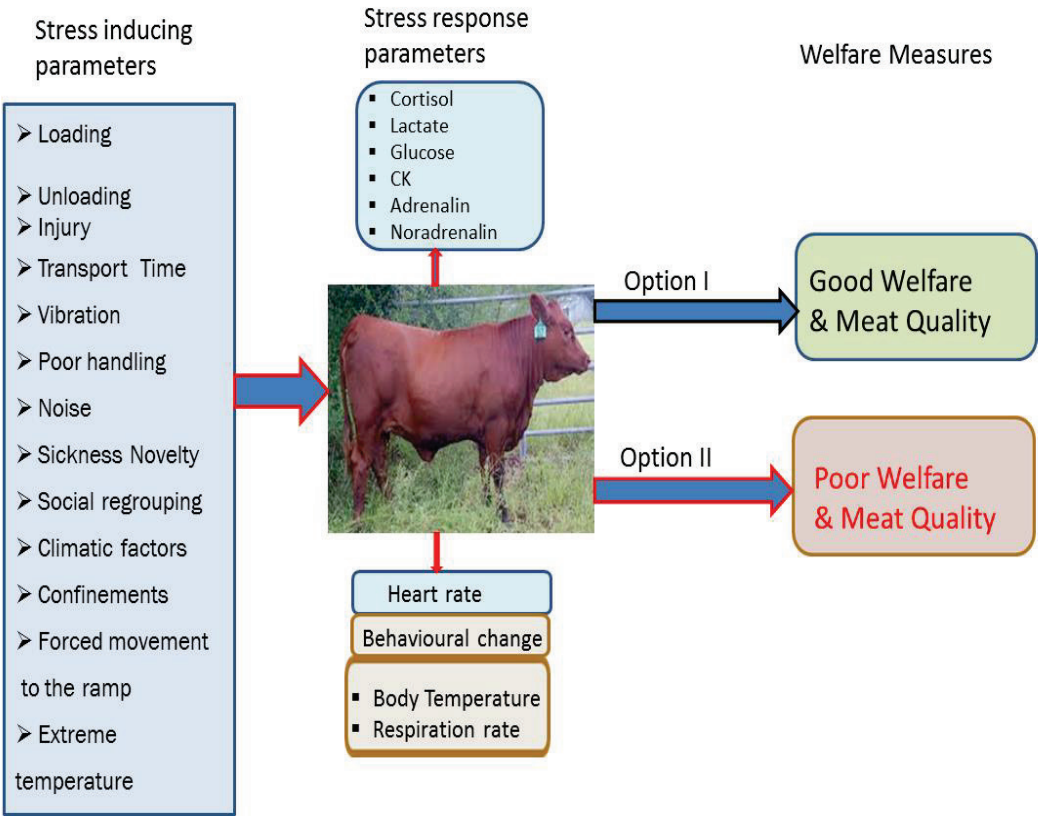


***Figure: 1*. Stress and welfare conditions of animal**

Stress is the total response of an animal to environmental demands or pressures that cause the body to release stress hormones. There are different types of environmental conditions which can cause stress in animals, a number of which are related to transport (Figure 1). These are vibration, loading, unloading, transport time, poor handling, sickness, noise, novelty, social regrouping, climate factors, and pre-transport management, feed and water deprivation. Stressors are stimulating factors that create stress responses. Stress response parameters, such as blood parameters (cortisol, lactate, glucose and Creatine kinase), heart rate and behavioural changes, body temperature and respiration rate, indicate the level of stress created and how the welfare of an animal is affected. The fact that animal welfare is affected by transport processes (transport time, loading, unloading and other transport-related activities) is evidenced by the physiological and behavioural changes observed in animals during transport. Stressors that trigger a physiological response may be long-term or short-term. When animals are stressed, heart rate increases from its resting condition to its peak value. If the animals adapt to the new conditions and have time to recover, their heart rate decreases to the resting level (see Figure 2). If the animals do not adapt, their heart rate remain at its peak point and the animals remain under stress conditions (Gebresenbet *et al*., 2006).

As shown in Figure 2, loading is the most stressful operation during transport and is mostly affected by ramp angle or inclination (Gebresenbet *et al*., 2006, 2012). However, there is a need for more knowledge on the dynamic behaviour of heart rate during loading.

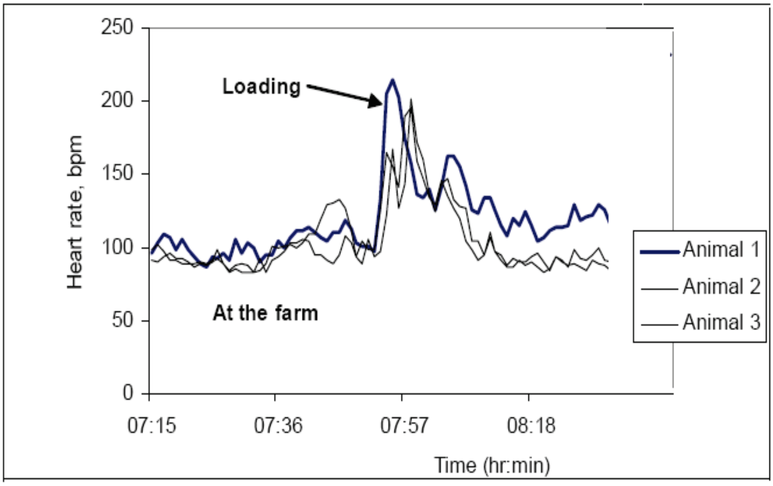


***Figure: 2*. Animal heart rate variation due to induced stress (Gebresenbet *et al.*, 2006)**

Transport processes, 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 remained undetermined. Recent research has indicated that vibration during transport has an impact on animal health, comfort and postural stability (Randall *et al*., 1995).

1.2. Literature review

Animals are transported by different means for different purposes. During animal transport to market or the abattoir, poor handling and long transport time and distances impose stress on animals. Animal responses in terms of physiological and behavioural alteration are indicators of stress levels induced by the external world (Gebresenbet & Sölvik, 2006). In the assessment of welfare, it is important to identify or consider ways of adapting to an unknown environment for animals and selecting less pressure that makes it free from disturbances, with major consequences for the changes in behaviour (Broom, 2006). Transport of animals has increased dramatically in recent decades, on both national and international level, in the pace with structural adjustment, specialisation of production systems, internationalisation and globalisation of marketing system (Gebresenbet, 1998). Animal transport needs more attention to ensure that the transport facilities used at national and international level are based on the findings of scientific research.

Cattle are transported by road, rail, sea and air for the purposes of breeding, fattening and slaughter (Grandin, 1978). Rough handling or poorly designed transport conditions determine both animal welfare and meat quality. According to this author, the important points to be considered when performing research are 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 animals. One study found that during transportation of pigs, the concentration of some blood parameters such as Creatine kinase exhibited a sharp increase after 1 hour and this elevated level was maintained during 2 hours of transportation (Yu *et al*., 2009). When animals are transported by vehicle, the flooring in the vehicle must be non-slippery, cleanable, disinfectable, sufficiently drained or free from urine/water to reduce injury and number of fallen animals (Gebresenbet *et al*., 2010). Transporting animals using vehicles not specifically designed for transport purposes leads to stress, suffering and injuries. Trunkfield and Broom (1990) discuss in detail the adverse effects that transport has on the welfare of animals. They provide evidence of changes in heart rate, mortality rate, enzyme levels, meat quality and behaviour. According to Lambooij *et al.* (1993), transport as a whole can induce stress in pigs, with climate conditions, loading density, duration of transport, cold draughts, heat stress, social stress, vibrations and noise all affecting the condition of the pigs during transport. Pigs are easily affected animals during transport because of their body structure and thus they need special care and managements.

Nowadays, animal welfare problems receive great attention and scientific research activities are increasing. Stress response in animals is adaptive to a certain degree, but when the stress level passes a certain threshold, animals do not adapt and enter a stressed state (Gebresenbet *et al*., 2010). The strength of stress can be evaluated by measuring stress response parameters such as blood hormones, heart rate, respiration rate and animal behaviour. To accurately assess an animal's reaction, a combination of behavioural and physiological measurements provide the best overall measurement of animal discomfort (Grandin, 1997). In vehicles, adequate ventilation and protection from temperature extremes (very high or low) during transport are very important for reduction of poor welfare. The animal stress reaction to handling procedures like transportation depends on three important factors: genetics, individual differences and previous experiences (Grandin, 1997). According to that author, facility design can have a strong influence on experience and poor design and planning are the main causes of increased stress levels. In all transport processes, the animals may be subjected to environmental stresses such as heat, cold, humidity, noise, motion and other stresses caused by social regrouping. According to Gebresenbet *et al*. (2005), conditions and processes preceding transport, such as preparation, planning, loading, management and unloading at the end of the transport chain, need to be optimised to improve the welfare of animals and meat quality. The welfare of animals concerns not simply stress experienced by an animal, but its ability to manage stress, whether physical or mental stress.

1.2.1. Heart rate modelling

The transportation of animals by road is a relatively recent practice that is increasing rapidly in countries all over the world. Animals react to any unfamiliar situations and do not adapt to procedures that are aversive to them. Heart rate is a sensitive response, reacting quickly and differently in individual animals. Heart rate is an important parameter to describe animal response to both emotional and physical stress and is frequently used as a sign of an autonomic response to stress and to measure the welfare of animals during exposure to acute stressors (Fraser & Broom, 1990). It is also used as measure of welfare for short-term stress. According to Rubio *et al*. (1989), heart rate is related to body weight. A variety of factors, such as prior experience, genetics, age, sex and even physiological condition shape the nature of an animal's affective response to a stressor (Moberg, 1987; Geers *et al*., 1995). Heart rate helps the researcher to observe rapid stress reactions that differs depending on the level of stress imposed to the animals. In heart rate modelling systems, mathematical modelling is a powerful approach to understand the complexity of biological conditions. Time-variant multivariate techniques are able to perform autoregressive spectral estimation and decomposition into components on a beat-to-beat basis rather than longer time intervals. In one study, the heart rate peak and the percentage of the peak heart rate in relation to the maximum heart rate predicted for the subject's age during a cardiopulmonary exercise test were found to be the same for optimised and non-optimised low sensibility (Carvalho *et al*., 2009). Modelling the neuron autonomic regulation of heart rate was used as a stochastic feedback system successfully accounts for the key characteristics of heart rate variability (Luis *et al*., 1999). The latter reported that the nature of heart rate is not a stable quantity, since even at rest it shows variability (increases or decreases). A heart rate (HR) variable always lies within certain physiological limits (between HR_{min} and HR_{max}). Non-linear model structures provide a more complete description of the heart rate system dynamics than linear structures. During transport, animal heart rate increases because of loading, unloading, high stocking density, vehicle speed variation and mixing of unfamiliar animals.

The above literature has contributed to existing knowledge on the variability of heart rate. Heart rate is an important parameter to be considered in animal transport, detailed studies are necessary, particularly during loading, the most stressful operation during transport.

1.2.2. Vibration

Vibration is a complicated phenomenon and animal exposure is difficult to investigate in a comprehensive way. Vibration on a load platform depends on road surface, speed and suspension system. The discomfort created by vibration transmission on animals during transport increases with the length of exposure time. The vehicle's motion and vibration are known to have effects on the health, comfort and postural stability of animals (Randall *et al*., 1995). Gebresenbet and Eriksson (1998) performed comprehensive field measurements on tri-axial vibration using a commercial animal transport vehicle, taking into consideration road conditions and speed variations. The most dominant frequencies they identified were 2, 4, 8 and 12 Hz. However, those investigations were made on relatively good (asphalted) roads and vibration levels also need to be 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 content and is represented by different frequency weightings. 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 *et al*., 1986).

In a study by Scott (1994), the fundamental frequency of poultry transport was between 1 and 2 Hz, with a secondary peak of 10 Hz and a chassis vibration in the lateral axis of 12-18 Hz. That study also found that standing birds maintained stability by wing extension and by flapping or squatting. Most of the impact of vibration is associated with transportation, where animals are exposed to mechanical disturbances during transport. Animals' response to vibration depends on the magnitude, frequency and duration of exposure. 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 changing as a result (Scott, 1994). Vibration, noise and handling are novel to animals and constitute a potential stressor. Vibration is a stress-inducing factor in animals as it emanates from the structure of the vehicle, road conditions, vehicle speed and driving performance of the driver. When high levels of vibration are transmitted to the animal's body during transport, they cause muscular fatigue and disturbances. Adjustment of vehicle suspension and upgrading the performance of drivers can improve the welfare of animals.

According to Perremans *et al*. (1996), heart rate measurements during vibration are dependent on body weight. Those authors also found that root mean square (RMS) acceleration had a significant effect on maximum heart rate. As indicated above, during transport vibration has an effect on the behavioural and emotional conditions of the animal above a certain level. Road type also can affect the level of vibration, which in turn affects animal welfare. Kenny and Tarrant (1987c) found that 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). Vibration occurs when a system is displaced from a position of stable equilibrium. Random vehicle vibration and shock can occur as a result of road surface irregularities.

Previous research has shown that the degree of discomfort and levels of vibration experienced by animals during transport while standing on the vibrating floor of vehicles subject them to swaying and loss of balance. These findings confirm that transport can be considered an acute stressor, causing physiological and behavioural stress in animals. In general, the effect of vibration on animals has not been studied in sufficient detail.

1.2.3. Transport time

The process of transport and length of transport time play an important role in animal welfare. Transportation duration causes stress in cattle that may alter physiological variables, with a negative impact on production and health (Murata *et al*., 1991). During animal transport, physiological, psychological and physical stress results in the release of hormones. As transport time and distance increase, animals become increasingly exhausted, dehydrated and stressed. Tadich *et al*. (2005) found a high Creatine kinase level in animal upon arrival at the abattoir after a transport time of 16 hours, but without any further increase during 24 h of lairage time. Creatine kinase is a blood hormone that indicates increased muscle fatigue because of restlessness and loss of balance behaviour during animal transport. Warriss *et al*. (1995) indicated that a journey of varying length (up to 31 hours) could compromise the welfare of cattle. They also provided evidence that although 15 hours of road transport was acceptable in terms of animal welfare, the cattle were becoming physically fatigued at the end of the journey. Knowles *et al*. (1999) noted that after transport of steers 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 from onset of transportation of up to 24 hours in a study by (Buckham Sporer *et al*. 2008). The cortisol concentration in that study reached its peak value at 14.25 hours before returning to the basal concentration at 24 and 48 hours. Cortisol concentration in cattle decreases over time as animals adapt to the situation during repeated exposure to transportation. According to (Wikner *et al.*2003) report the effects of various climate conditions, stocking density and transport times has sever effect on animals during transport. To transport animals in a safe way and within an appropriate time, attention and monitoring by the handler and transporter of animals is important. Animals that are less exposed to handling become more stressed during transport. The welfare of animals can be compromised if the environment is new to the animal. Long time and long distance transport is the main cause of dehydration and loss of live weight in transported animals.

In general, the effect of duration of transport and its effect on pig and cattle welfare have not been studied in detail and needs further investigation.

1.2.4. Animal transport by trekking

Transport causes physical and behavioural problems because farm animals are not accustomed to transport conditions and procedures during their early life. The movement of animals by walking is still used in most developing countries (see Figure 3) and is particularly stressful for the animals. Moving animals by walking is suitable only where roads and other infrastructure do not exist or when distances from farm to destination are short. This mode of moving animals is full of risks and has a negative impact on the welfare of the animals. According to (Ndou *et al*. 2011), in the developing world, where food insecurity and poverty are prevalent and the welfare of animals receives low priority due to factors such as traditional customs and beliefs, lack of knowledge and low attention are given to animal handling facilities. Animal welfare conditions can be measured by various techniques to determine animal health, behaviour and physiological responses. According to Ayo and Oladele (1996, *cit*. Minka, 2007) in tropical Africa the majority of animals are transported commercially under adverse climate conditions, over very long distances of 2-3 days' journey. During transport, natural animal behaviour with one following the other can create calm movement. Marketing of animals and animal products is the main livelihood of farmers and traders in Ethiopia, as animal producers or farmers sell their animals and animal products to cover their needs and household cash expenses. Live animals are marketed in the traditional marketing system, which has been employed and known for a long time (Tegene *et al*., 2006). The welfare of animals can be compromised up to the point of death if the animal cannot cope with its new environment. The term welfare is relevant only when an animal is alive, but death during handling and transport is usually preceded by a period of poor welfare (Broom, 1993).

Whenever possible, walking animals should be moved at a normal walking speed, while acclimatising the animals to handling and close contact with people reduces stress (Grandin, 1997a; Fordyce, 1987; Boandl *et al*., 1989). Fraser (2008) noted that in Africa and Asia, large numbers of animals are driven on foot for many days to market or slaughter with little food and water. Research clearly shows that animals which are handled in a negative manner and fear humans have lower weight gain, fewer offspring (pigs), and give less milk (cattle) and reduced egg production (laying hens) (Hemsworth, 1981; Barnett *et al*., 1992; Hemsworth *et al*., 2000). Animals show responses indicative of stress during and after transport. If the animals are stressed before and/or during slaughter, this not only affects animal welfare but can also have unwanted consequences for the meat quality (Gregory *et al*., 2010). To evaluate animal welfare, behavioural measurements are among the preferred methods, since the animals behave in response to the new environment (Broom, 2007). Furthermore, physiological responses such as heart rate and blood hormones are good indicators to study animal welfare. In developing countries including Ethiopia, during animal transport by walking/trekking transported animals are subjected to long-distance journeys, forced to cross big rivers that have no bridge and have to complete the journeys without sufficient food, water and resting time, causing stress to animals. Furthermore, the animals are exposed to high temperatures and heavy rain, both during transport and at abattoirs. The stakeholders (farmers and traders) who participate in the animal trade and transport chain are not educated or trained for their job and have insufficient knowledge and understanding about the welfare of animals. In general, poor animal welfare results in loss of weight, physical injuries, sickness and sometimes even death of animals.

Figure: 3. **Animal transport from Gudar to Finfinnee city market in Ethiopia by trekking/walking.**

Animal transport by walking/trekking is also used to transport live animals from producers to the next market level. In most developing countries the transport of such animals is mainly by walking, or by substandard vehicles not designed for animal transport (Kenny & Tarrant, 1987). According to Gebremedhin (2007), almost all livestock in Ethiopia are transported by walking. Despite this dominance of transport by trekking/walking in most developing countries, including Ethiopia, little information is available as regards its effect on animal welfare. Animal welfare conditions are affected because of the absence of loading facilities on farms and by transport on hot days or in hot, wet weather. As mentioned above, in developing countries, transported animals are subjected to long distance and time transport without rest, food and water, with high stocking density and sunburn. It is important to note that most previous researchers have not used stress hormones and behavioural parameters simultaneously to gain a comprehensive understanding of animal welfare in such conditions.

2. Objectives and structure of the thesis

2.1. Objectives

Important research related to animal handling, transport and welfare has already been conducted, but many questions remained to be addressed, particularly on the effect of transport time, marketing, vibration and climate conditions. The aim of this thesis was to assess a system for animal transport processes and handling in relation to welfare.

Specific objectives were to:

a. Investigate the dynamic performance of heart rate responses in heifers and cows during loading for transport and describe the heart rate time series using a dynamic simulation model.

b. Determine the vibration levels and frequencies of a typical vehicle used for cattle transport and 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, cows and bulls transported under conventional conditions in terms of thermal stress, stress hormones, behavioural alteration and pH values.

d. Investigate the animal handling and welfare issues during transport to market with particular focus on cattle flow to and from Gudar livestock market and the activity chain of Ambo abattoir in Ethiopia.

2.2. Structure of the thesis

This thesis is based on the work described in Papers I-V. The relationship between these papers is shown in Figure 4. The overall outcome of the thesis work could lead to improvements in the animal welfare during handling, transport and marketing.

Paper I helps to provide a good understanding of the dynamic performance of heart rate responses of heifers and cows during loading for transport and describes the heart rate time series using a dynamic simulation model. Paper II considered vibration levels and frequencies of a typical vehicle used for cattle transport and the vibration level transferred from vehicle to animals during transport. Papers III and IV examined the effect of transport times of up to 12 hours on the welfare of pigs, cows and bulls transported under conventional condition in terms of thermal stress, stress hormones, behavioural alteration and pH values. Paper V investigated animal handling and welfare issues during transport for marketing in a developing world situation, with special focus on cattle flow to and from Gudar livestock market in Ethiopia.

The papers provide an overview of the state-of-the-art in animal transport and handling, the effect of stress-inducing factors and response parameters in transported animals. Papers I-IV mainly focused on animal transport by vehicle and the effect of loading, driving, unloading, air quality and vibration on animal welfare during transport in Sweden. Paper V focused on animal transport to and from Gudar livestock market and the operations of Ambo abattoir in Ethiopia.

The stress response parameters that were considered for each paper are listed in Table 1. In general, animals suffer from pre-slaughter stress that arises from bruises, injuries, starvation, tiredness, and water and food deprivation, loading and unloading onto and off vehicles. This thesis work was intended to improve the welfare of transported animals by identifying ways to improve handling methods, transport conditions and processes.

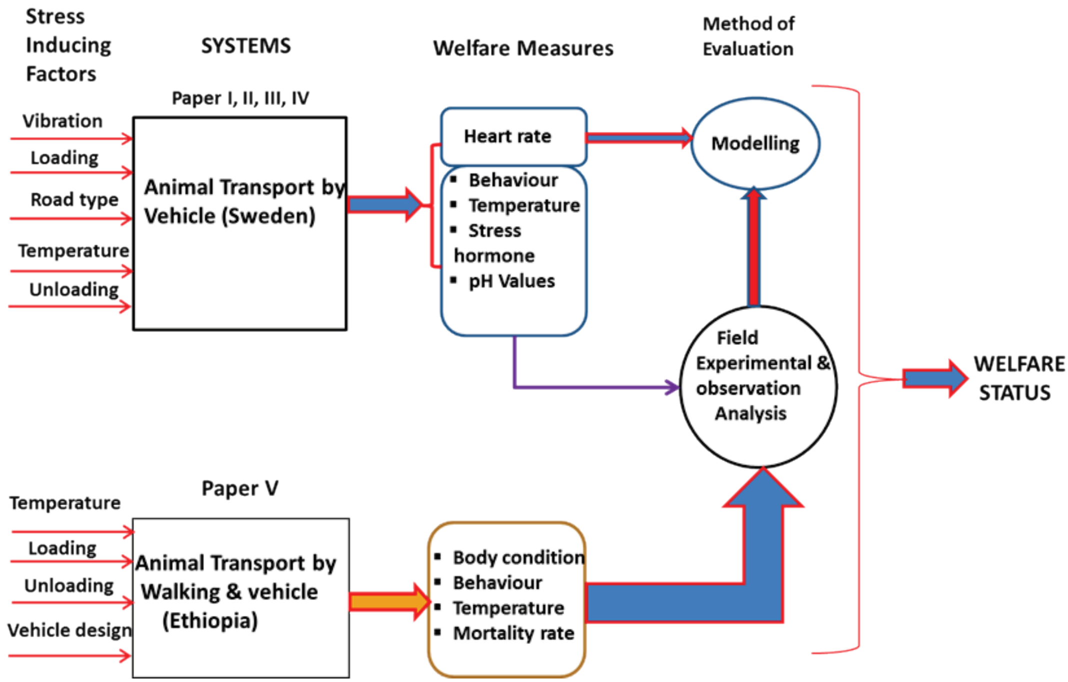


Figure 4: **Structure of the work performed in Papers I-V of the thesis**.

3. Methodology

The main methods used in Papers I-V includes field measurements, data analysis and simulations, interviews with the help of a questionnaire and field observations.

In Paper I, which involved modelling, field experiments were carried out using 18 cattle (11 heifers and 7 cows). These cattle were transported from two farms to the Uppsala abattoir. The instrument package manufactured by Polar Electro Oy Finland (Gebresenbet *et al.*, 2010) was used for heart rate measurement. Two parameters, rising rate (r_1) and recovery rate (r_2), were used to describe the increase and subsequent decline in heart rate with the Power Opt software. The model simulates the heart rate of individual animals at any time within the time window of the loading activity.

In Paper II, the vibration study in the field, five cows used for each trip and a typical cattle truck was used for transport. The vehicle had a single deck with two pens separated by a steel gate and an air suspension system fitted to the chassis. The vehicle was fitted with vibration sensors to measure vibrations on the vehicle and the vibration transmitted to the animals. Three road types, four speeds and two animal standing orientations with two repetitions were performed. The analyses were carried out using Mat lab software (version R2009b). Statistical data analysis was also conducted with SAS software, using the General Linear Model (GLM) and analysis of variance (ANOVA) procedures.

In Paper III, which examined the effects of transport duration, 2753 pigs were transported from various farms to the abattoir. Of these, 216 were transported in an observation crate or box and behavioural studies were performed on them. Blood samples were collected from 90 pigs during the field experiment, and blood samples were also collected for control purposes from 20 pigs that were not in transit. The average age of the pigs was 6 months and their average weight was 100 kg. Statistical data analysis was performed separately for blood parameters, behaviour, pH and air quality using the SAS 9.2 PC-based statistical package. Multivariate analysis was also performed using GLM, multivariate analysis of variance (MANOVA) and clustering (dendrogram).

In Paper IV, also on transport duration**,** a conventional cattle transporter was used for the experiment and the observation pen was 2.50 m x 2.45 m. Sensors to measure temperature, relative humidity and a camera to monitor behaviour were mounted in the pen. Eighteen measurements were performed during summer and winter for 4, 8, and 12 h transport time, with three replications. For pH determination, meat samples taken from the longissimus dorsi were chilled at 4 ^{o}C for 24 h. To determine cortisol, glucose, lactate and Creatine kinase concentrations, blood samples were collected before and after transport from 80 bulls, 82 cows and 20 control animals.

In Paper V, which studied animal transport by walking and handling during marketing, the study area was Gudar livestock market, located in Oromiyaa Regional State, Ethiopia. Data were collected through interviews and questionnaires from key informants such as farmers, traders, and workers at the Ambo abattoir, tax collectors, butcheries and institutions.

3.1. Parameters

In the thesis work, to measure blood parameters, blood samples were taken at the farms before transport and at the abattoir immediately after stunning for determination of concentrations of blood hormones. For meat pH value, meat samples were taken from the longissimus dorsi. Cow, bull and pig behaviours were continuously observed and documented at farms in transit and during unloading at the abattoir. The trucks used for cattle and pig transport were equipped with adjustable loading ramps. Temperature and relative humidity inside the container of the vehicles were measured simultaneously and continuously throughout the transport period. Stress-inducing and response parameters associated with transport activities were used to evaluate animal welfare and meat quality (see Table 1). These parameters and factors such as transport time, temperature, relative humidity, loading and unloading conditions, vibration, speed, behaviour, number of stops, space allowance and total number of animals in the vehicle were considered.

Table: 1. Stress-induced and response parameters considered in each of Papers I-V

Stress parameters considered	Paper-I	Paper-II	Paper-III & IV	Paper-V
Stress induced parameters	Loading	Road condition Speed Vibration Standing orientation	Transport time Temperature Relative humidity	Transport time Loading Handling Social regrouping
Stress response parameters	Heart rate	Physiological stress Emotional stress Behavioural change	Stress hormones pH and Behavioural change	Physical stress Emotional and Thermal stress

3.2. Heart rate measurement during loading

The cattle were transported from farms to Uppsala abattoir and were of the Swedish red breed. Measurement of heart rate started before loading to obtain data on heart rate in resting conditions and continued until the end of loading activities. A Polar transmitter with elastic belt and Polar heart rate monitor devices were used to measure the dynamic responses in the heart rate of cattle during loading for transport. The heart rate recording process was started by mounting the sensor on the chest of the animal as shown in Figure 5 and heart rate data were recorded and transferred to the computer for analysis. The heart rate data were recorded a beat-to-beat basis.

Figure: 5. **Sensor and receiver used for recording the heart rate of cattle during loading for transport**

3.3. Modelling of heart rate

The dynamic response of heart rate of cattle during loading was modelled and studied. To describe the pattern of heart rate response in cattle, a mathematical exponential function was used. The Powersim simulation software (Powersim Corporation, 1997) which utilises the system dynamics method was used to model heart rate of cows and heifers. The mathematical expression used to describe the pattern of animals' heart rate signals during loading was:

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

Where HR (t) is heart rate at time t, HR_{rest} - heart rate at rest, HR_{max} - heart rate at maximum, HR_{rec} - heart rate during recovery, A_1 - rising amplitude, A_2 – recovery amplitude, T_1 –rising period, T_2 – recovery period

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

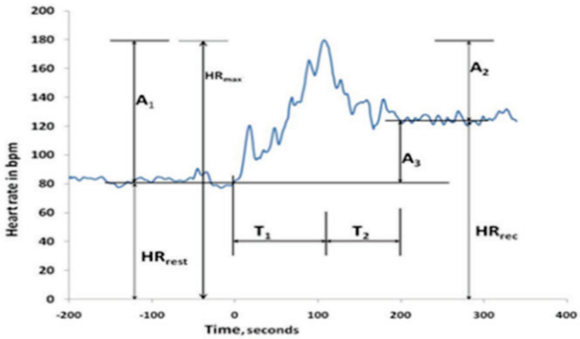


Figure: 6. **Heart rate responses of cattle during loading for transport**

This exponential function was used to describe the pattern of heart rate during loading and the Powersim software (Powersim Corporation, 1997) was used to build a simulation model. Measured parameters such as heart rate-related parameters (heart rate at rest, heart rate at maximum, heart rate during recovery and rising/and falling period) were obtained from recorded data (Figure 6). Parameters such as rising rate (r_1) and recovery rate (r_2) were determined using PowerOpt, a software package that works interactively with Powersim software. 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 for each animal involved in the experiment, *i.e.* 18 data sets were used (Paper I).

3.4. Vibration measurement and analysis

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

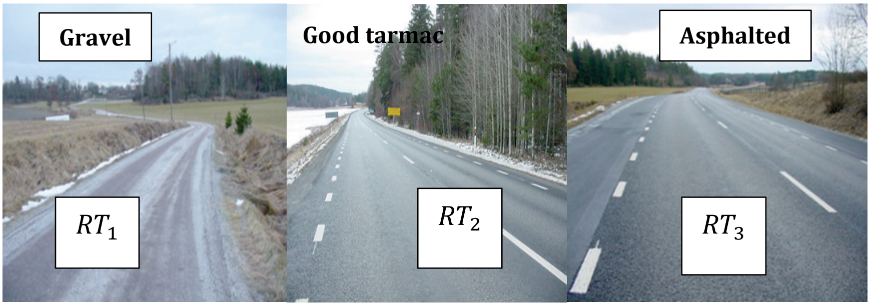


***Figure:** 7.* **Road types used during the experiment: gravel ($RT1$), good tarmac ($RT2$) and asphalt ($RT3$).**

Vibration levels were measured at two positions on the vehicle, using vibration sensors mounted on the chassis and floor as shown in Figure 8a. These sensors were connected to the computer by cable. Vibration levels on the animals were measured in three directions (acc_1-acc_3 sensors) using a tri-axial accelerometer and loggers as shown in Figure 8b. The logger containing sensors was mounted with tape on a girth belt around each animal's chest cavity. On each trip, measurements were made on five animals simultaneously. Each measurement was triggered manually via the cab computer and data were 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-s periods on animals $acc_3$with the same sampling frequency as the acc_1 and acc_2 sensors.

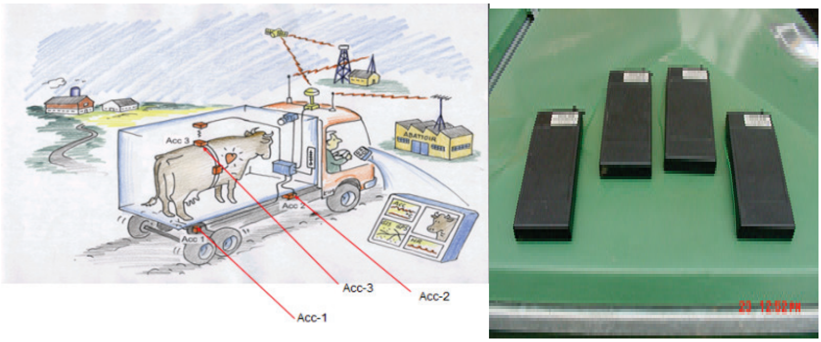


Figure: 8. **(a) Measurement sensors mounted on vehicle and animal to measure vibration levels and (b) loggers used to record vibration levels (diagram taken from Gebresenbet, 1997).**

Parameters such as root mean square (RMS), crest factor (CF), vibration dose value (VDV), estimated vibration dose value (eVDV), transmissibility and power spectrum density (PSD) were evaluated. All analyses were performed using Matlab software (version R2009b). Statistical data analysis was also conducted with SAS software, using the GLM and ANOVA procedures.

Acceleration values were measured and used to estimate transmissibility from chassis to floor of the vehicle and from floor to animal, while RMS and VDV were used similarly to evaluate the level of transmissibility. European Council Directive 2002/44/EC (EC, 2002) specifies a daily limit value for exposure to vibrations of 1.15m/s^2 and a daily exposure action value of 0.5 m/s^2 for an 8-hour reference period. Based on these values, vibration exposure was determined for 8 hours of transport time using frequency-weighted RMS from measured data calculated according to the following equations:

- In vertical direction $a_x\,(8) = a_{wx}\sqrt{\frac{T_{exp}}{T_0}}$.............. ...... (1)

- In horizontal direction $a_y\ (8) = 1.4\ a_{wy} \sqrt{\frac{T_{exp}}{T_0}}$ .... (2)

- In lateral direction $a_z\ (8) = 1.4\ a_{wz} \sqrt{\frac{T_{exp}}{T_0}}$ .... (3)

where T_{exp} is exposure time to vibration during transport, T_0 is total transport time, a_{wx}, a_{wy} and a_{wz} are frequency-weighted root mean square acceleration in the vertical, horizontal and lateral directions, and 1.4 is a multiplying factor only for the lateral and horizontal axes.

Analysis of vibration parameters

The main factors that determine the discomfort of an animal exposed to vibration are vibration magnitude, frequency, direction, point of entry and duration of exposure. The CF 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 RMS acceleration value (a_w). Crest factor indicates a high impulsive level of shock and it can be calculated as (Griffin, 1990):

$$\text{CF} = \frac{\max(a_w(t))}{a_w} \quad \ldots\ldots\ldots\ldots (4)$$

Where CF is crest factor

Transmissibility is used to describe the effectiveness of a vibration isolation system, expressed as the ratio of input to output (Griffin, 1990).

$$Transmissibility = \frac{a_i\,floor}{a_i chassis} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$Transmissibility = \frac{a_i\,animal}{a_i floor} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Acceleration on the chassis was used as input with respect to floor and floor acceleration was used as input with respect to animal acceleration. The parameter estimated vibration dose value (eVDV) was calculated to assess the net vibration absorbed by the body during the period of exposure while VDV, a cumulative measure of vibration, was used to determine the total severity of vibrations. Vibration dose value (VDV) and estimated vibration dose value (eVDV) were determined according to Griffin (1990):

$$\text{VDV}= \left[\frac{T_S}{N}\sum a^4(i)\right]^{\frac{1}{4}} \ldots \ldots\ldots\ldots\ldots \quad (7) \quad \text{And}$$

$$\text{eVDV}= \lceil(1.4R)^4 T_S\rceil^{\frac{1}{4}} \ldots\ldots\ldots\ldots\ldots \quad (8)$$

Where $T_S\ is\ samplingperiod, a(i)$ - 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

Transportation is a strange and critical situation in animal life. In Papers III and IV, measurement and analysis of stress hormones were performed to study the effect of transport time on animal welfare. Cortisol, Creatine kinase, lactate and glucose were the parameters considered and blood samples were taken on the farm before transport and after unloading at the abattoir. In all, blood samples were taken from a total of 90 pigs, 82 cows and 80 bulls for the determination of concentrations of stress hormones and every animal was bled twice (on the farm immediately before the start of transportation and immediately after transportation and subsequent stunning at the abattoir). Blood samples were taken from the jugular vein of pigs and the coccygeal vein of cows and bulls.

The cortisol values in blood of transported and control pigs or cattle were measured using radioimmunoassay Coat-A-Count cortisol kits. Serum glucose and Creatine kinase were analysed using an automatic Konelab analyser and lactate levels were measured using a GM7 Analox analyser. Blood samples were also taken from pigs and cattle that were not transported. The values obtained from these control pigs and cattle 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 of pigs and cattle for pH determination. The carcasses were chilled for 24 hours at +4 °C before sampling. In addition, the decrease in temperature and pH were measured in the longissimus dorsi between the 12th and 13th rib immediately after slaughter, and at 5, 18 and 24 hours post-mortem.

3.5.3. Behavioural parameters

The behaviour of pigs and cattle was continuously observed and documented on the farm (during blood sampling and loading), during transport (in the vehicle) and on unloading at the abattoir, by visual observation and by using 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, the 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 is* occurrence of behaviour, *B is* total number of pigs or cows and bulls in the observation box, and *t* is duration of events in minutes

To evaluate the welfare of pigs and cattle during transport, stress-inducing factors associated with pre-loading, loading, transport and unloading were considered. The study was carried out in a farm-transport-abattoir system. Statistical data analysis was performed separately for blood parameters, behaviour, pH and air quality.

3.6. Animal transport by walking in Ethiopia

The study area in Paper V was Gudar livestock market located in Oromiyaa regional state of Ethiopia. Figure 9 shows the animal flow from origin or farms to feeder markets (Finchahaa, Shaambuu, Baakoo, Shobokaa, Daannoo, Noonnoo, Geedoo and Xiqurhinchinni) and then transport to Gudar regional market only by walking. After their arrival at Gudar market, animals were grouped into four groups: G_1, G_2, G_3 and G_4 (see Figure 9 for detailed information). Data and information were collected through interviews and questionnaires. The study mainly focused on selected stakeholders' farmers and traders. Traders were categorised into Category 1 or Category 2. In order to investigate the occurrence of incidents such as death and injuries of animals during transport, more detailed information was gathered from 21 of the traders who purchased animals from feeder markets and transported them to Gudar market and then on to Finfinnee city market, which is the final destination.

In addition to conducting interviews, observations were made on animal conditions and handling at the market, as well as during loading activities. In addition to animal flows to and from Gudar livestock market, the location of Gudar livestock market and some feeder markets is shown in Figure 9.

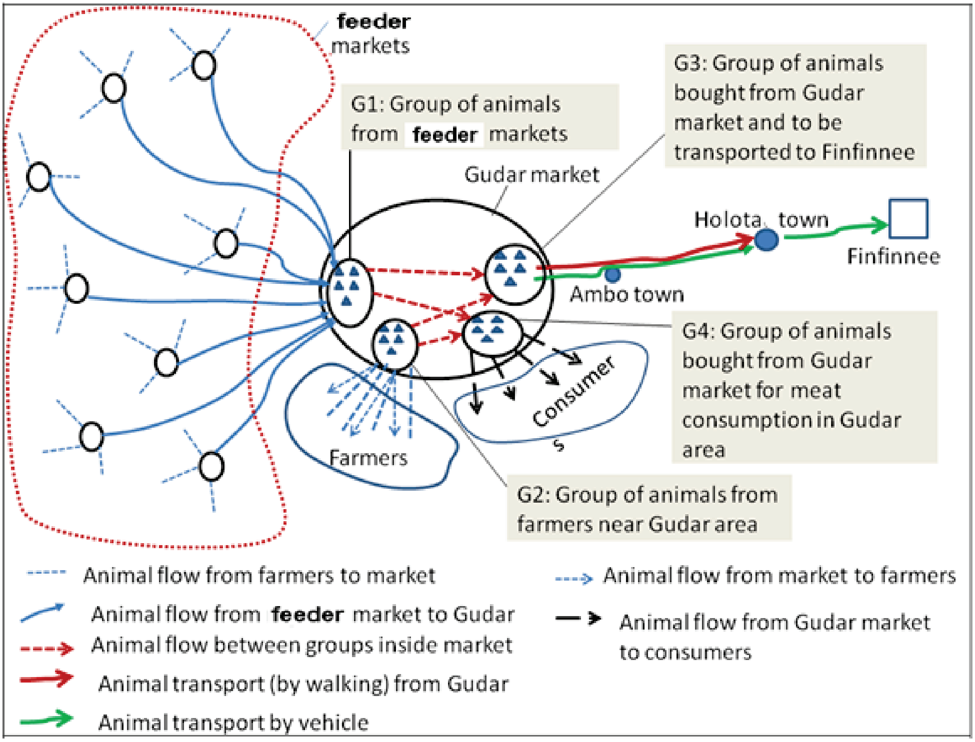


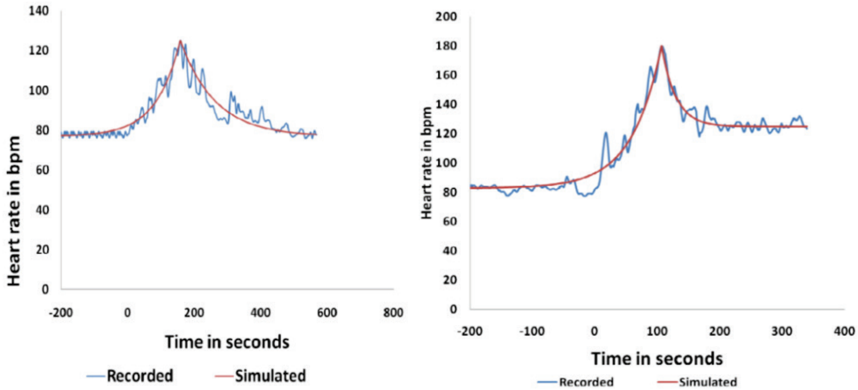
***Figure: 9*. Supply and animal marketing chain for Gudar market in Oromia regional state, Ethiopia.**

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 stresses. The heart rate variability of cows and heifers was observed as it increased exponentially from resting level to peak value and decreased slowly from the peak point to the recovery level (Figure 10). It was found that the difference between rising and recovery amplitude increased as the rising amplitude increased. The difference between the rising and recovery amplitude showed that the animal stayed under stress conditions, *i.e.* heart rate did not fully recovered to its resting level (Figure 10b). However, the smaller the difference between the rising and recovery amplitude, the less stress level or the more the animal adapted to the new environment. The relationships between rising rate (r_1) and period (T_1), recovery rate (r_2) and period (T_2) were investigated and identified. The values of r_1 and r_2 decreased as T_1 and T_2 increased. In general, it was noted that the mean value of rising rate was nearly twice the recovery rate value for both animals studied; this showed that the heart rate rose more rapidly and recovered slowly.

The recorded and simulated curve of two types of animals (cows, heifers) is shown in Figure 10 to illustrate the heart rate pattern during loading. Figure 10a shows when the heart rate is fully recovered, while Figure 10b indicates partially recovered heart rate of the cows and heifers during loading for transport. The model simulates the pattern of heart rate response. During simulation, it was observed that the simulated values of HR $_{rest}$, HR $_{max}$ and HR $_{rec}$ were almost the same as the recorded values for each subject.

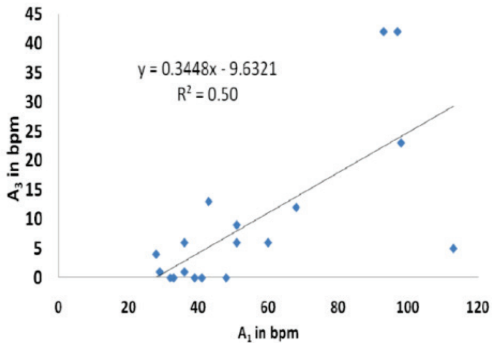


(a) **(b)**

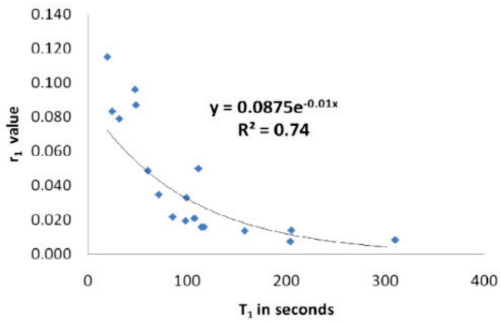
Figure: 10. **Simulated and recorded heart rate pattern during loading of cows and heifers (a) when the heart rate had fully recovered and (b) when the heart rate had partially recovered.**

The mean heart rate at resting condition, peak, and after recovery was 80 ± 6 beats per minute (bpm), 136 ± 35 bpm and 91 ± 19 bpm for heifers and 47 ± 4 bpm, 102 ± 27 bpm and 55 ± 12 bpm for cows. It can thus be concluded that mean value of heart rate in heifers is higher than in cows. In general, during stress the heart rate was rose exponentially from its mean resting value to peak value at about 1.9 times the resting value. During the recovery period, heart rate declined and maintained steady state at the HR_{rec} value, about 1.15 times the resting value, on average. The simulated data were directly correlated with the recorded data (coefficient of determination(R^2) = 0.89 ± 0.06). the pattern of heart rate response and the mean value of R^2 were similar for cows and heifers, with no significant differences. Considering all together, for the heifers and cows the amplitude A_1 was 55 ± 27 bpm and A_2

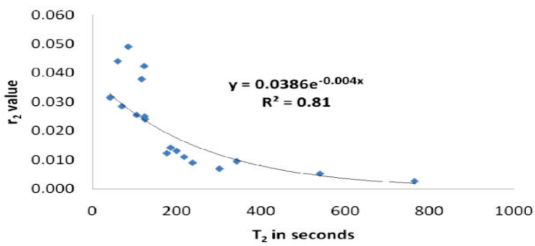
was 46 ± 20 bpm. A_3, Showed the difference between the rising and falling amplitudes, as its value varied from 0 - 42 bpm. The relationship between A_1 and $A_3$was such that A_3 increased with an increase in A_1 (Figure 11a). A high A_3 value suggests that the animal was under more stressed conditions and heart rate did not fully recover to the resting level (Figure 10b).



(a)



(b)



(c)

Figure: 11. **Relation between parameters (a) The relation between A_1 andA_3 (b) The relation betweenr_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 Paper II, the results showed that the transmissibility of vibration level from the chassis to the floor damped as the vibration level from the vehicle floor to animal was amplified. Detailed results are presented in Table 2. The highest level of vibration recorded on animals was on gravel roads at driving speeds of 50 km/h and 70 km/h in the driving direction.

Table2**.** *Transmissibility of acceleration (a), vibration dose value (VDV) and root mean square (RMS) from vehicle chassis to floor and from floor to cattle*

	Vehicle Floor/ Chassis			Cattle/Vehicle floor		
Parameter	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.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

Vibrations in the horizontal and lateral directions were lower on animals positioned perpendicular to the direction of travel than on those facing forward. Road conditions ($P<0.0002$) and standing orientation ($P<0.002$) both had a significant effect on vibration levels. Measurements of VDV, eVDV, RMS and CF during transport on tarmac roads revealed that 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 ($4.25 \pm 0.61$ to $7.96 \pm 2.36\frac{m}{s^{1.75}}$) and RMS ($0.81 \pm 0.12$ to $1.52 \pm 0.45\frac{m}{s^{2}}$) was higher on the chassis than on the vehicle floor and cattle. Furthermore, VDV, eVDV and RMS 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 (Table 3).

Table: 3**.** *Measured crest factor (CF) on chassis, vehicle floor and animal in three orthogonal axes (x, y, z)*

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

The smallest CF value was found for cattle, rather than vehicle, and it was 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 was observed on tarmac road with a speed of 85 km/h.

4.3. Effects of transport times up to 12 hour on welfare of pigs

4.3.1. Stress hormone parameters

The concentration of cortisol was significantly ($P<0.001$) elevated during short transport time and the rate of elevation decreased with increased transport time (Figure 12). The rate of elevation during winter was 58.2-25.3 nmol/L while during summer it was 59.2-31.8 nmol/L.

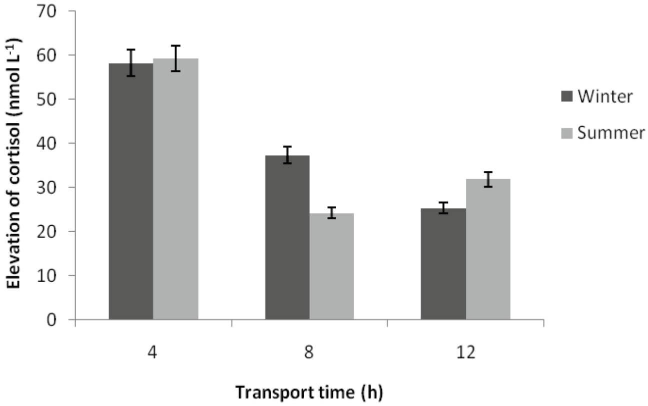


Figure: 12. **Mean and standard deviation of cortisol concentration in pigs during winter and summer for 4, 8 and 12 hours of transport**

Glucose concentration increased from short to medium transport time and decreased thereafter. Glucose concentration was highest during winter for 8 hours of transport and lowest during summer for 12 hours of transport time (Figure 13). During the 8 hour transport time, the maximum concentration noted were 20.46 mmol/L, which was three-fold the reference value. The effect of transport time on concentration of glucose was significant ($P<0.01$).

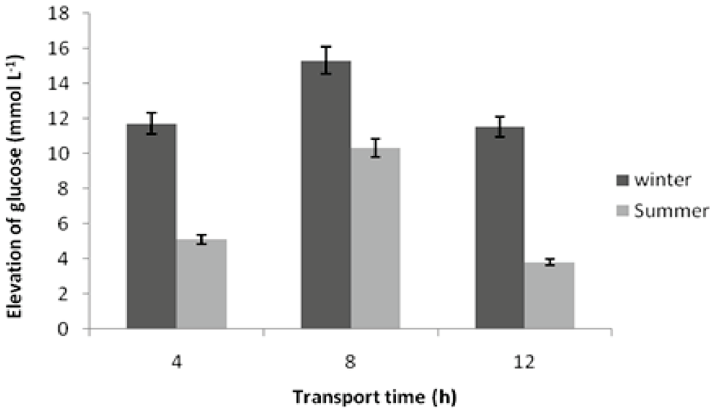


Figure: 13. **Mean and standard deviation of glucose concentration during winter and summer for 4, 8 and 12 hours of transport**

Lactate concentration was positively correlated with transport time and increased as transport time increased (Figure 14). Concentration of lactate varied between 4.2 and 7.3 mmol/L. Blood concentration of lactate during winter increased from 4.7 to 6.2 $\mathrm{mmol/L}$ with an increase in transport time, with a positive correlation ($R^2 = 1$; $P<0.002$) between these parameters.

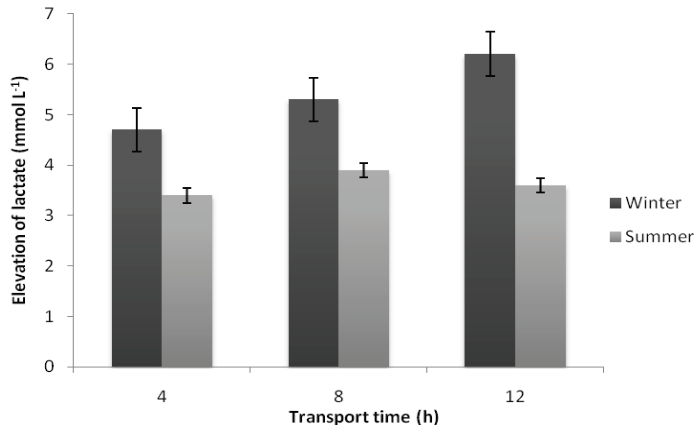


Figure: 14. **Mean and standard deviation of lactate concentration during winter and summer for 4, 8 and 12 hours of transport**

Concentrations of creatine kinase were also positively correlated with transport time (Figure 15). The concentration of creatine kinase ranged from 0.4 to 25.4 $\mu\mathrm{mol/L}$ during winter and from 2.5 to 31 $\mu\mathrm{mol/L}$ during summer and was thus positively correlated with transport time ($P<0.002$). There was also a positive correlation between creatine kinase concentration and transport time for summer and winter seasons combined ($R^2 = 0.99$). The rate of increase in creatine kinase concentration from 4 hours to 8 hours of transport was lower than that from 8 hours to 12 hours of transport (Figure 15). During the summer season and with 12 hour transport time, the maximum value after transport was 154 $\mu\frac{\mathrm{mol}}{\mathrm{L}}$, which exceeded the reference value of 129 $\mu\mathrm{mol/L}$. Creatine kinase concentration for winter and summer increased exponentially with transport time.

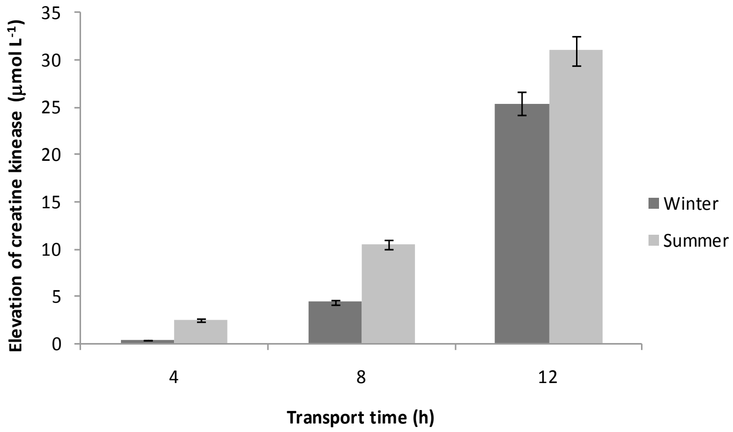


***Figure: 15*. Mean and standard deviation of creatine kinase concentration during winter and summer for 4, 8 and 12 hours of transport**

4.3.2. Behavioural alteration and quantification

The behaviour of animals can change in response to many environmental difficulties and it was used in this thesis to identify stress in response to transportation. The final quantification of behaviour was expressed as the product of frequency of events and duration of events. Lying, sitting, rooting, vocalisation, restless and change of position, smelling, panting, loss of balance and fighting in pigs were found to be significantly and positively correlated with transport time ($P<0.009$) (Table 4). Values for rooting and vocalisation were higher during loading. Behaviours like rooting, reversal and vocalisation showed that the severity of stress was higher during loading than unloading.

Table: 4. *Observed behaviours by pigs at 4-h intervals within 12 hours of transport*

Behaviour	Transport time (h)					
	0 – 4		4 - 8		8 - 12	
	Freq	Freq x time	Freq	Freq x time	Freq	Freq x 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	2.1	403.8	4.43	614.57
Pt	0	0	0.2	0.48	0.3	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	7.4	0.77	5.25	0.52	3.02
Vc	2.2	3.81	1.8	2.73	1.1	2.52

Where Ear erecting (Er), Fighting (Ft), Loss of balance (Ls), Jumping one on the other (Jn), Lying one on another (Ln), Lying (Ly), Panting (Pt), Rooting (Rt), Smelling (Sm), Sitting (St), Restlessness & change of position (Rc), Reversal (Rv), Vocalization (Vc)

Lying behaviour in relation to transport time showed interesting results. During all experiments, about 50% of pigs were lying about 2 hour after the vehicle started moving. Then about 80% of pigs were lying after 6 hour transport time, and the behaviour increased in similar way until 12 hour (Figure 16).

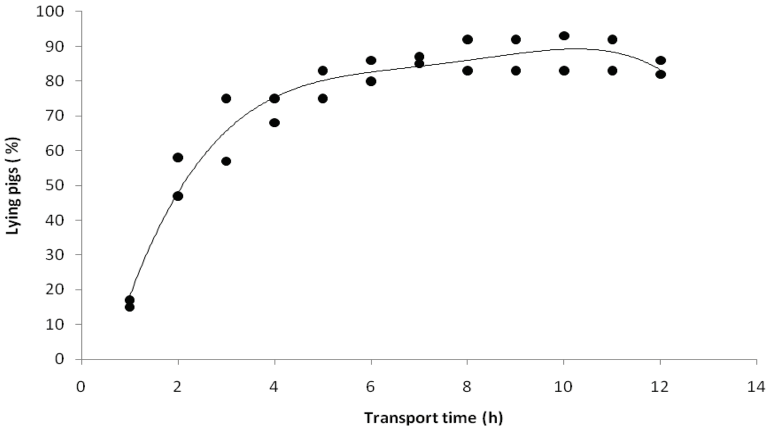


Figure: 16. **Proportion of pigs lying in percentage depending on transport time, described with 4th order polynomial function, with correlation coefficient, r^2 = 0.95**.

4.3.3. pH value

In Paper III, the lowest (5.5) and highest (6.1) pH values were recorded for 4 hour and 12 hour transport time, respectively. The highest pH_{24} value in the pigs, 5.99 ± 0.29, occurred during summer with the 12 hour transport time (Figure 17 and Table 5). The measured pH_{24} values in carcases showed positive correlations with transport time during summer. The mean pH_{24} value during summer season for 12 hour transport time exceeded 5.8, and this elevation in pH_{24} might be attributable to climate conditions in the vehicle. The pH_{24} values during summer were correlated with transport time (R^2 = 0.69)

(Figure 17). During summer, loading caused thermal stress, while inadequate ventilation after washing was the main cause of high initial relative humidity.

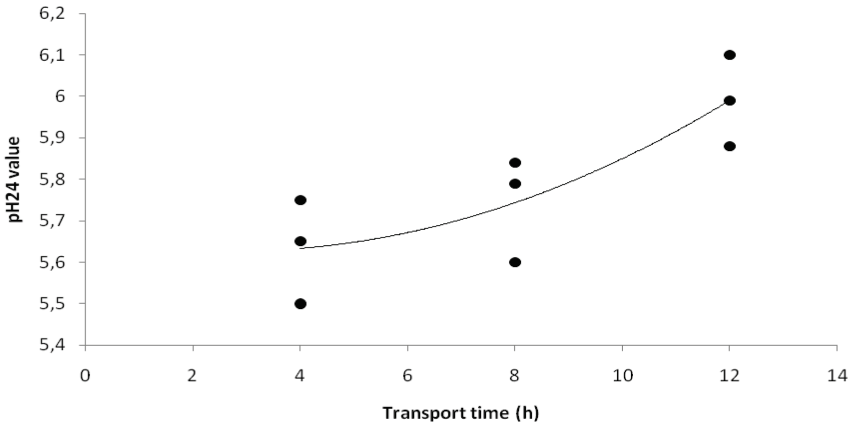


Figure: 17. **Effect of transport time on pH_{24} value in pig carcases during summer (correlation coefficient r^2 = 0.69).**

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

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

A summary of the mean pH values measured in pigs is presented in Table 5. The highest pH_{24} value for winter was 5.58 ± 0.14. As Table 5 shows, there were also seasonal effects on pH values at pH_0, pH_5, pH_{18} and final pH at different hour's post-mortem. During winter measurements, the values of pH_0 and final pH ranged from 6.5 to 5.5, whereas summer pH values varied between 6.29 and 5.99. The final pH values for the 4, 8 and 12 hours of transport are also shown in Table 5.

4.3.4. Temperature and relative humidity

Measurements of temperature and relative humidity were performed simultaneously and continuously throughout the transport time and the temperature-humidity index (THI) was calculated. It is a single value that 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 and used to assess heat stress in the animals.

Table: 6. *Recommended temperature-humidity index* (*THI) thresholds for farm animals (source: CGIR)*

THI	Heat stress category
≤ 74	Normal
75-78	Alert
79-83	Danger
≥ 84	Emergency

Table: 7. *Summary of mean values of temperature, relative humidity and temperature-humidity index* (*THI) in winter and summer for different transport times of pigs*

	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	94.56	65.92

The mean THI values in both seasons and for the three transport times (4, 8 and 12 hour) were below the threshold value of 74 (Table 6) and the maximum mean value was 65.92 (Table 7). Regarding the relationship between THI and transport time, seasonal variation had more effect than transport time. The THI values were used to describe categories of heat stress associated with hot weather conditions. Certain THI values during summer were above the normal (≥ 74) level during loading. The overall THI mean values for 4, 8 and 12 h transport time for two seasons remained below the threshold value considered as normal. The THI in 4-hour intervals (0-4, 4-8 and 8-12 h) of 4, 8 and 12 hour transport time was investigated by considering the maximum, minimum, mean and standard deviations of values of each interval. Based on the recommended threshold value of 74, the peak THI value was found to be in the danger zone (79.24) during the 12 hour transport time.

4.4. Effect of Transport time up to 12 hour on Welfare of Cows and Bulls

4.4.1. Stress hormone parameters

Stress can be categorised as physiological, emotional or a combination of these. Stress is the sum total of all emotional and physical input to the animal for a given period of time. The animal body's stress response system is usually dependent on the level of animal resistance. The concentrations of cortisol, glucose, lactate and Creatine kinase in cows and bulls for all transport times studied during the two seasons in Paper IV are summarised below.

4.4.1.1. Cortisol

In both bulls and cows during all seasons, the concentration of cortisol was significantly elevated (P<0.001) during short transport and the rate of elevation decreased with an increase in transport time. The rate of elevation was generally inversely proportional to transport time for the range of transport times used for this experiment (Figure 18). However, in bull's cortisol concentration during 12 hour transport in summer did not decrease with transport time.

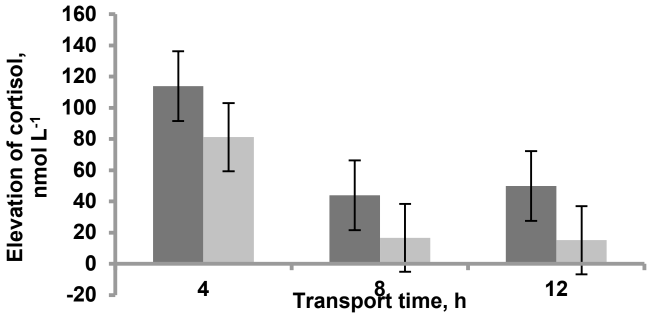


Figure 18: **Mean and standard error of cortisol concentration in cows for the different transport times and seasons dark and grey colours represent winter and summer, respectively**

4.4.1.2. Glucose

The effect of transport on concentration of glucose was significant (P<0.01) for both bulls and cows. However in cows, during both seasons, the highest elevation was observed at 4 and 12 hour and the lowest at 8 hour transport time (Figure 19).

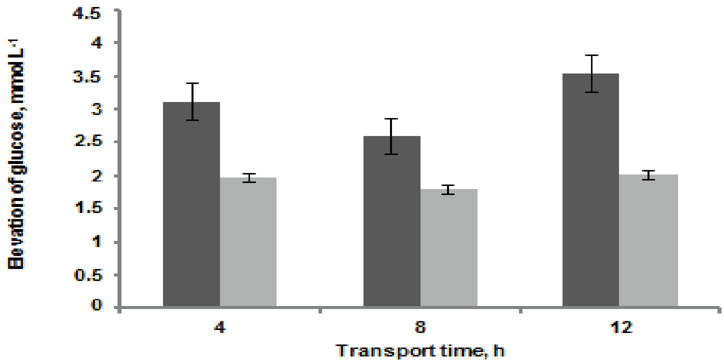


Figure 19: **Mean and standard error of glucose concentration in cows for the different transport times and seasons dark and grey colours represent winter and summer, respectively.**

In bulls, glucose concentration increased with an increase in transport time (p<0.02) and the relationship remained the same for both seasons (Figure 20). Glucose concentration was elevated by 1.52-2.78 mmol/L, with the maximum value observed being7.33 mmol/L.

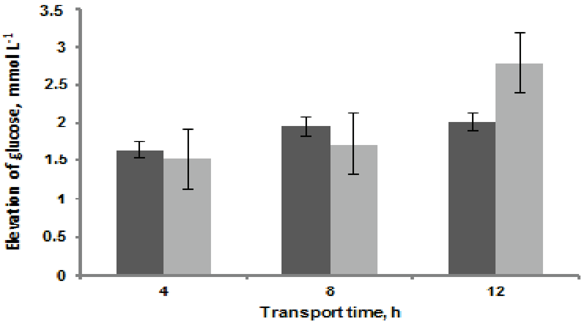


Figure .20. **Mean and standard error of glucose concentration in bulls for different transport times and season's dark and grey colours represent winter and summer, respectively**

4.4.1.3. Lactate

Lactate concentration in the blood increased with an increase in transport time in cows and it was positively correlated with transport time ($P<0.001$) during summer and winter. Concentration of lactate in winter increased in a linear way ($R^2 = 0.98$) (Figure 21).

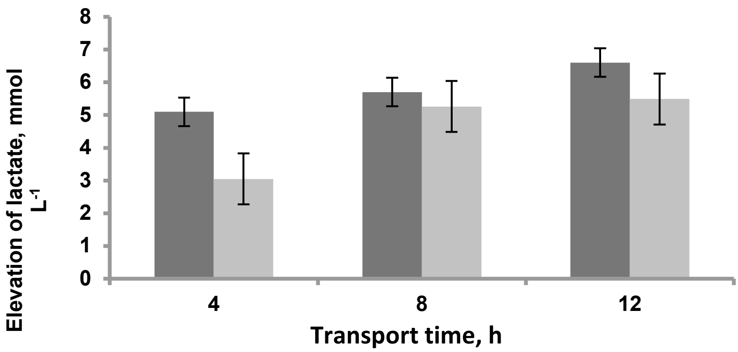


Figure 21. **Mean and standard error of lactate concentration in cows for different transport times and seasons dark and grey colours represent winter and summer, respectively**

In bulls (summer), the concentration of lactate remained the same for 4 and 8 hour transport, while it increased by about 25% for 12 hour transport (Figure 22). In winter, the concentration increased with an increase in transport time from 4 to 8 hour, and decreased thereafter with an increase in transport time from 8 to 12 hour.

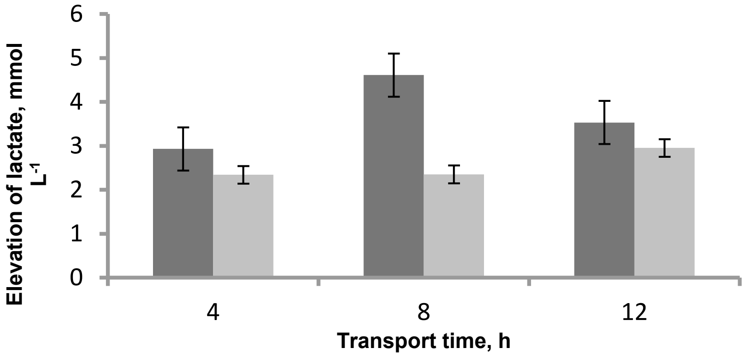


Figure 22. **Mean and standard error of lactate concentration in bulls for different transport times and season's dark and grey colours represent winter and summer, respectively**

4.4.1.4. Creatine kinase

In cows, both during summer and winter, the concentration of Creatine kinase increased with an increase in transport time (from 4 to 8 hour) and remained at a steady state or slightly increased thereafter (Figure 23). The highest measured value, for 12 hour transport time, was 109µmol/L.

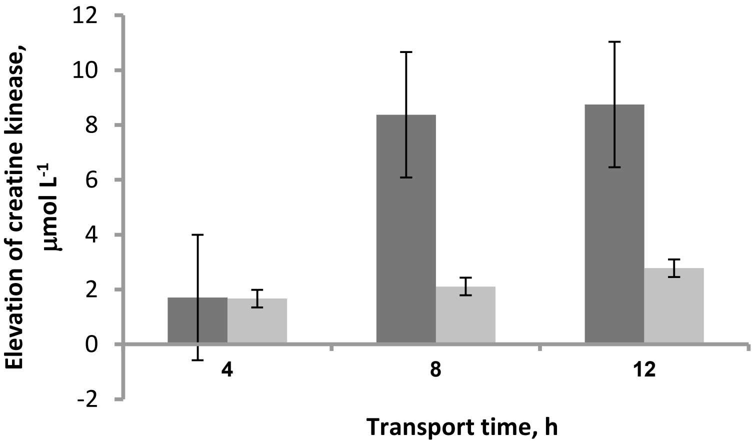


Figure 23. **Mean and standard error of Creatine kinase concentration in cows for different transport times and seasons dark and grey colours represent winter and summer, respectively.**

In bulls, the rate of increase in concentration of Creatine kinase was different during winter and summer. There was slight increase from 4 to 8 hour transport, but from 8 to 12 hour the increase was as much as three-fold. The increase in concentration at winter measurement was best described in coefficient of exponential form ($R^2 = 0.8556$). During summer measurements, the concentration of Creatine kinase increased by about four-fold from 4 to 8 hour transport and maintained nearly steady state between 8 and 12 hour transport time (Figure 24). The maximum value observed (for 12 hour) during winter was 98 µmol/L.

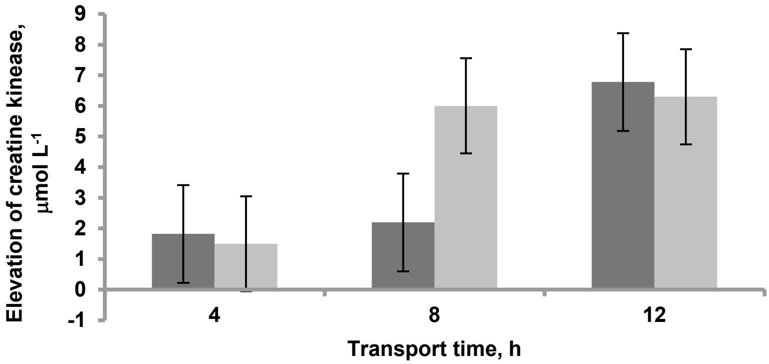


Figure 24. **Mean and standard error of Creatine kinase concentration in bulls for different transport times and seasons dark and grey colours represent winter and summer, respectively.**

In both cows and bulls, transport had a significant effect on Creatine kinase concentration ($P<0.001$) and was positively correlated with transport time.

4.4.2. Behavioural alteration and quantification

Behavioural stress is an indicator of discomfort during animal transport. Behaviours such as turning, mounting, fighting, change of position and lying are limited to loose cattle, because the movement of tied animals is restricted. The total number of observed animals in Paper IV was 162, and 12% of these animals (cows and bulls) were tied. Behaviours of animals were quantified as described earlier. The abnormal behaviours demonstrated by the animals that were recorded during the observations were different in pattern and frequency from the normal behaviour.

During loading, which involved separation of animals from familiar groups and places, certain abnormal behaviours were observed. Elimination (El), smelling (Sm), vocalisation (Vc), aggressiveness (Ag) and refusal to mount the ramp (Rm) were the main behaviours exhibited by both bulls and cows. However, reversal (Rv) was a behaviour exhibited only by bulls. When the animals were unloaded, the most frequently observed behaviours were elimination (El), smelling (Sm), aggressiveness (Ag), refusal to go down the ramp (Rd) and vocalisation (Vc). 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 and increased with transport time. In both types of animals, swaying, loss of balance and restlessness and change of position were positively correlated with transport time.

4.4.3. pH value

High pH value and depletion of muscle glycogen are the result of long-term stress or chronic stress. Ultimate pH and muscle glycogen are also indicators of physiological fatigue and stress in animals. The results obtained in Paper IV for cows in winter and summer with 12-hour transport time and the corresponding pH values are presented in Figure 25. The results show that long journeys led to higher ultimate pH in the longissimus dorsi muscle. The initial pH values for winter and summer were 6.47 and 6.42, respectively, and the pH_5 value for winter (5.74) was higher than for summer (5.61). The ultimate pH value for both winter and summer was 5.5.

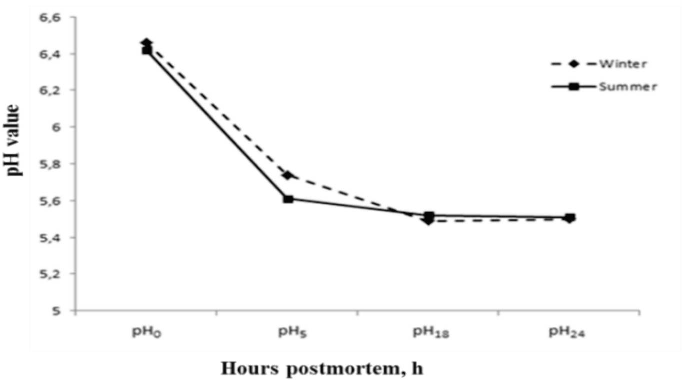


Figure 25. **Typical pH values in cow carcases during summer and winter after 12 hours of transport.**

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 in the study as a whole, transport time had no significant effect on pH_{24} values in cows and bulls.

4.4.4. Temperature and relative humidity

In Paper IV, the lowest temperatures were recorded in March (-3 °C) and the highest in June (23 °C). Transport time (8 hour), temperature and relative humidity variations are illustrated in Figure 26. This was an event where the temperature dropped below 0 °C and varied between -0.5 and -1.5 °C during transport. During loading, the temperature was 12 °C and it began to decrease immediately after the vehicle started moving.

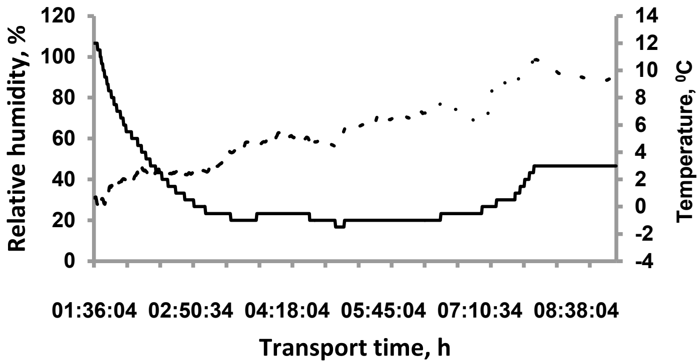


Figure 26. **Temperature and relative humidity variations during 8 hours of transport bold lines represent temperature and dashed lines relative humidity.**

As can be observed in Figure 26, the temperature was already -0.5 °C after about 1.5 h of transport. During the whole observation period from farm to abattoir, the temperature variation was between -3 and 12 °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 temperature was below 0 °C relative humidity continued rising, from 44 to 74.3%.

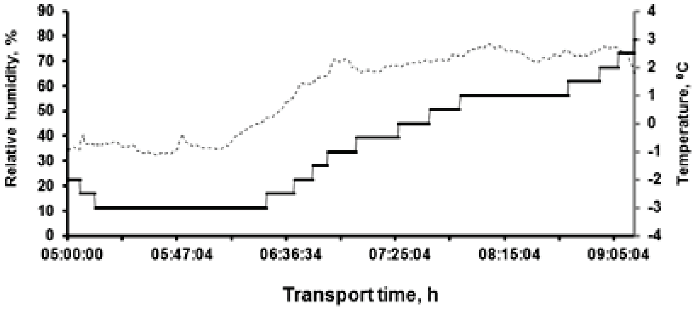


Figure 27. **Temperature and relative humidity variations during 4 hours of transport bold lines represent temperature and dashed lines relative humidity**

.Figure 27 depicts the changes in temperature and relative humidity during the 4 hour transport time. The initial temperature at the time of loading was -2 °C and it continued falling to -3 °C during transport. The total time in which the temperature remained below zero was 2: hour and 30 h. During loading, initial relative humidity was 35%. Despite the decline of temperature described, relative humidity kept on rising to a level of 70.7%. The relative humidity also continued rising when the temperature started rising and reached 75%, and thereafter maintained a steady state. During the whole trip, temperature varied between -3 and 3 °C (see Figure 27).

Figure 28 illustrates typical temperature and relative humidity curves during summer for 12 hour transport time. In this case temperature at loading stage was around 22 °C, and decreased gradually and thereafter maintained steady state. However, when extra animals were loaded at about 17.00 hours, the temperature started rising and continued until unloading and reached about 18 °C. During loading, relative humidity was 39% and the increase was gradual. During the trip, relative humidity varied between 39 and 86%.

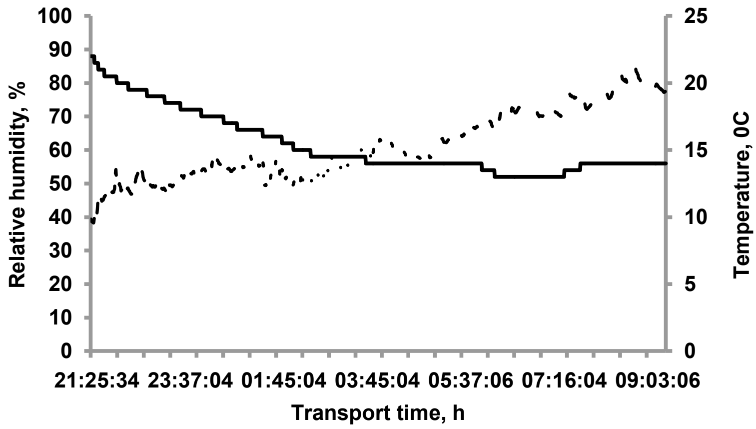


Figure 28. **Temperature and relative humidity profile for 12 h transport time (bulls). Bold lines represent temperature and dashed lines relative humidity.**

In general, the rate of increase in relative humidity and temperature from initial values to maximum values depended on number of animals in the vehicle, initial value of relative humidity prior to loading and length of loading time. These factors had a significant effect on rate of increase of relative humidity, regardless of length of transport time.

Temperature-humidity index is used as weather safety index and to evaluate the combined effect of temperature and relative humidity. The THI values of all experiments are summarised in Table 8. Relative humidity reached its maximum value during both winter and summer experiments, but the THI mean values of winter and summer seasons and for the three transport times (4, 8 and 12 hour) were below the threshold value of 74 (see Table 7) and the maximum mean value was 65.92, which is considered normal weather conditions. For winter experiments, the lowest mean value of THI was 39.67 and the highest 45.95. The THI value generally describes the category of heat stress. Heat stress in animals can lead to changes in body temperature and expose them to dehydration.

Table 8: *Summary of mean values of temperature, relative humidity and temperature-humidity index (THI) observed in winter and summer seasons and for the three transport times (4, 8 and 12 h)*

Season	Animal category	Transport time, h								
		4			8			12		
		Temp ^0C	RH %	THI	Temp ^0C	RH %	THI	Temp ^0C	RH %	THI
Winter	Bulls	6.09	68.37	45.59	3.34	72.65	41.04	4.39	80.47	41.86
	Cows	4.33	75.15	42.30	7.45	92.25	45.95	7.1	97.4	44.97
Summer	Bulls	14.68	86.01	58.39	18.14	98.48	64.60	15.28	83.68	59.36
	Cows	17.05	90.93	62.45	16.83	98.84	62.27	14.56	88.84	58.19

4.5. Animal handling during supply for marketing and slaughtering

During transport, animals are subjected to stressors that compromise their welfare. Handling during transport is one of the main causes of stressors. Transport of cattle by walking between farm and slaughter house is undoubtedly the most stressful and injurious form of transport. In Paper V, the animals were transported from eight feeder markets to Gudar regional market after a long walk. These feeder markets are used as main suppliers for Gudar regional market. The study indicated that the flow of cattle to regional market and then on to central market (Finfinnee) consisted of walking, which took up to 4 days, or transport by vehicles, which took up to 3 hours. However, the vehicles used for transport are not designed for animal transport and there are no loading facilities, so animal welfare is compromised due to vehicle design and handling conditions.

4.5.1. Animals at Gudar market

Concern about the welfare of animals during transport is increasing as animal production and transport for slaughter and breeding is increasing all over the world. At Gudar market, the number of animals varied from one market day to another and the number of animals supplied to the market per market day was estimated to be between 1000 and 2000. The data collected during three consecutive market days showed that oxen were the dominant category, while heifers were the least frequently observed cattle category in the market. At Gudar market, the animals were first mixed and categorised into groups (Figure 9 Paper V). This grouping of animals could help to prevent transmission of diseases between animals and to make the market situation stable.

In Paper V, farmers were asked why they were selling their animals. The results showed that 26.1% of the farmers sold animals to buy fertiliser and 13% sold animals to pay the school fees for their children, to buy seeds and to cover payments for social activities or events, while the remaining 17.4% of farmers sold their animals to provide the basic necessities for their families (see Figure 29).

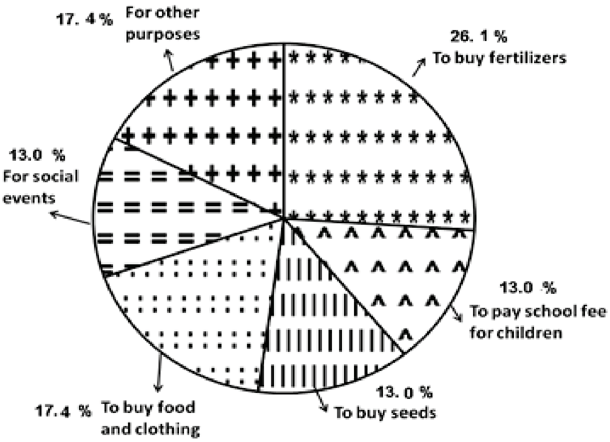


Figure: 29. **The reasons why farmers sale their animals**

Figure: 30. **Animals being transported from the feeder market to Gudar market by walking.**

Animals that were transported from the vicinity of Gudar to the market were transported an average of 13 km by walking, which took about 1 to 6 hours to cover the journey up to Gudar market (Figure 30). The distance covered by animals that were transported from farm/point of origin to final destination (Finfinnee market) was 369 km (319 km by walking) and the time taken was 100 hour (95 hour by walking).

Animal handling and other welfare-related problems observed in Paper V were lameness and injury to bone, muscle, swelling of leg and inflammation linked to infection during transport by walking. The animals were exposed to mishandling, car accidents, physical fatigue, extreme weather conditions, and lack of food and water and physical injuries. Of the total number of animals transported to Gudar market from feeder markets observed in Paper V, 7.6% died, 6.9% were injured and 2.8% were stolen on the way (Figure 31a). During animals were being transported by walking from Gudar to Finfinnee market (final destination), 16% died (including car accidents) and 10.7% were injured (Figure 31b).

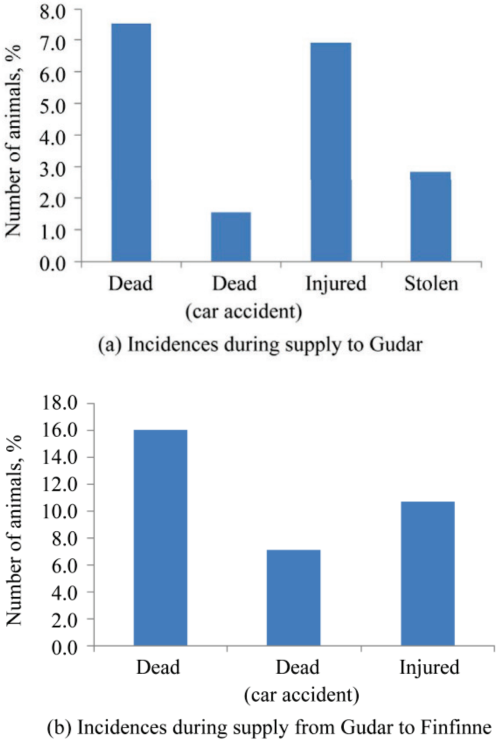


***Figure: 31*. Incidents leading to poor animal welfare situations during transport to market (a) Number of dead, injured and stolen animals during transport from feeder markets to Gudar market, based on about 318 animals sourced from 7 feeder markets by 21 traders per market day. (b) Number of dead and injured animals during transport from Gudar to Finfinnee market, based on about 56 animals purchased (by three traders) from Gudar per market day and transported to Finfinnee by walking.**

At Gudar market, during transport by vehicle, loading of animals for transport started by tying a rope to the animal's horns or neck and they were then strongly pulled towards the vehicle. When loading animals for transport, the loading ramp should be designed in safe way. In particular, the vertical distance from the floor of the vehicle to the ground must be suitable for the animal to mount the ramp. However, at Gudar the loading site was constructed by the workers, who had no knowledge of ramp construction, heaping and levelling soil. The animals were loaded forcefully and sometimes ran, vocalised and refused to climb into the vehicle. The animal loaders beat the animals repeatedly if they refused to climb, so loading was forcible (Figure 32).

Figure: 32. **Loading processes and site for animal transport at Gudar market**

The hilled and levelled loading ramp height was about 0.75 m high and measured 2.6 m by 1.5 m, which was not suitable for loading animals properly (Figure 32). During loading, when the animals were forced to climb into the vehicle compartment by their loaders (Figure 32), the lack of loading ramp exposed the animals to injuries and other uncomfortable conditions.

4.5.2. Ambo abattoir

Ambo abattoir has its own activity chain that is structured into different operations (Figure 33), but these differ from the standards imposed in developed countries, which involve animal delivery from market to stunning place, slaughter operation from stunning to evisceration, packaging and distribution of meat, delivery of hides to market, and waste disposal. Some of the animals delivered to Ambo abattoir were very exhausted, injured and had lost weight, mainly due to long journey and inhumane handling. The slaughter operations at Ambo abattoir were identical to the traditional way of slaughtering by local farmers, *i.e.* with no stunning or separation of activities, the only difference being that there was a veterinary inspection at the abattoir.

Ambo abattoir mostly provides services for butcheries, institutions, hotels and restaurants. The physical conditions that the animals showed at Ambo abattoir were loss of weight, physical injuries, dehydration, exhaustion and fatigue because of extreme weather conditions, food and water deprivation, long-distance journey and no rest time during walking.

Ambo abattoir lacks basic facilities present at most standard abattoirs. It has a traditional slaughtering system, has no cooling room and refrigerator storage, has no modern slaughtering machines, stunning and bleeding occur at the same place, there are no safety procedures for animals and all wastes, including blood, are discharged into a river which is for drinking purposes by residences of Ambo town and local farmers. As a result, it may facilitate the spread of disease.

The slaughter of animals in Ambo abattoir is carried out in an unsuitable slaughter house by untrained youngsters who have little awareness of sanitary problems. The stunning is done by the traditional method, with a knife in the animal's neck, and three animals are slaughtered at once. Carcasses are not held in refrigerated storage, but suspended in the traditional way on hooks. Waste products found at Ambo abattoir during the study period were dung, fat tissue, bone, intestine, blood, stomach, horn and hoof. Some of the solid waste products are used for dogs and cats as food. There are no special waste disposal systems or treatments. Dung and other wastes containing blood are discharged into the nearby river. Bones and hooves collected from the abattoir are sometimes burnt at the abattoir site, causing smoke with bad smell that could pollute the air.

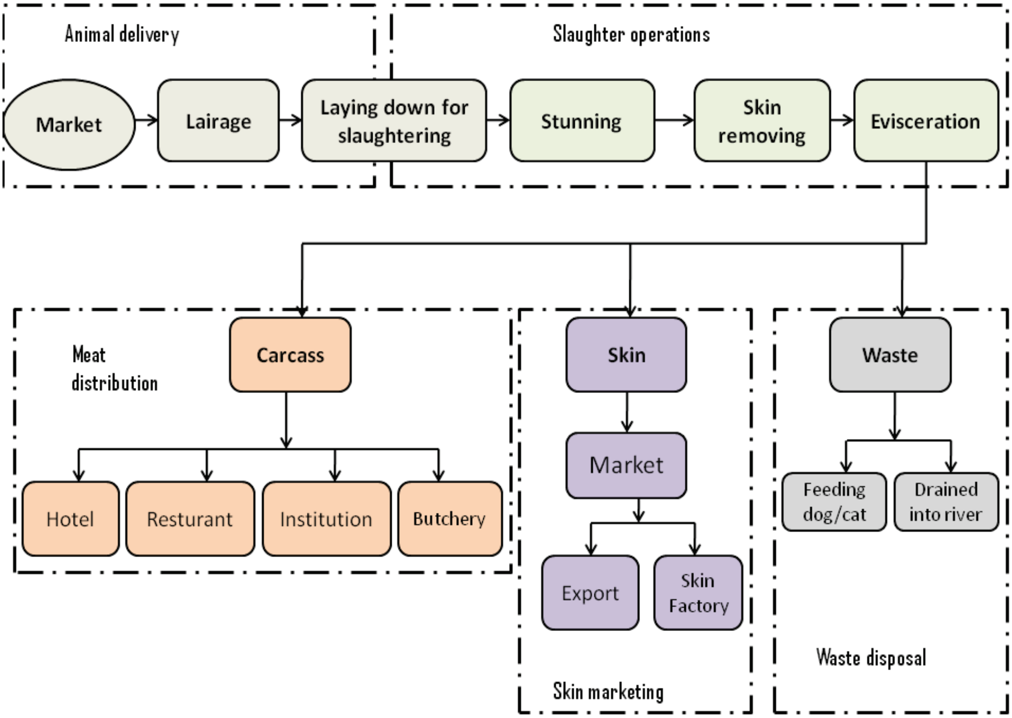


Figure: 33. **Operational structure of Ambo abattoir**

5. Discussion

5.1. General

Transport is unnatural and stressful for animals, and, like any other stressor, it should be minimised. The main variables to be controlled to reduce stress are transport processes, time and handling facilities. The physical condition of the animal before transport, and food and water deprivation during the journey play important roles in the way that animals should be managed during the transport process and in their fitness to travel. Handling, loading, transport conditions and unloading are critical processes that should be performed properly. Transportation of animals is associated with a number of handling and environmental stressors, such as vibration, climate conditions in the vehicle, thirst, hunger, humidity, high stocking density or overloading, inadequate ventilation and mixing of unfamiliar animals. As demonstrated in this thesis, transport is a major stressor that has effects on health, well-being, performance and meat quality. During long-distance transportation, animals are exposed simultaneously to a variety of stressors in a relatively short period of time (Grandin, 2000). Such stressors include fasting and water deprivation, mixing of unacquainted individuals, handling by humans, exposure to a novel environment, noise and vibration, forced physical exercise and extremes of temperature and humidity (Sainsbury & Sainsbury, 1988). In long and unsuitable journey situations, animals become exhausted, dehydrated and stressed, some of the animals are injured and some of the journeys are during summer in hot weather, with no adequate ventilation systems. These stressors compromise animal welfare and result in poor welfare and meat quality. The transport processes of loading, transport time, unloading and other transport-related activities as a whole affect animal welfare, as demonstrated by increases in the concentrations of cortisol, glucose, lactate and Creatine kinase and alteration of behaviour in all transport times considered during our study.

Welfare of animals could be improved if animal producers, handlers and transporters increased their knowledge of how animals are affected by being in different environmental situations. Stress in animals was successfully estimated in this thesis according to a set of behavioural indicators caused by stress-inducing factors. The results indicated that physical or emotional stresses had the overall worst effects on the animals. Behavioural changes in an animal can thus be used as an important indicator that an animal is experiencing difficulties in coping with the new aversive environment (Gebresenbet *et al*., 2012). Handling can be described as the activity of humans that involves how animals are touched, moved and interacted with during transport. Proper handling of animals during transport, marketing, before and during slaughter, including *e.g*. proper feeding and resting, can greatly reduce animal discomfort and stress. In this thesis, a comprehensive study was made covering the conditions of transport by vehicle, walking and vibration, where these factors were shown to have an effect on animal welfare. The most important stress-inducing factors considered were loading and unloading, vibration, transport time (by vehicle and walking), and climate conditions in the vehicle. The main stress response parameters considered were blood hormones, heart rate and behavioural alteration. The combination of these parameters was necessary to describe the welfare status of animals, as a reflection of physiological and behavioural condition and general well-being. An animal in a poor state of welfare suffers discomfort, distress, pain, injury, malnutrition and thirst, which may compromise its ability to cope with its environment. The five separate studies performed in this thesis (Papers I-V; see Figure 4) were designed to provide answers to the main question of what major parameters to consider to improve animal welfare.

Vibration and transport time are strongly linked to each other, because if animals are subjected to vibration for a long time, this could cause trauma in the animals. That is why it was considered important to study these parameters in Papers II-IV. According to Peeters *et al*. (2008)), the motion of the vehicle floor used for pig transport depends on road surface, vehicle suspension, load, floor rigidity, engine speed, transmission and wheel imbalance and the acceleration is higher on standing pigs than on lying pigs during transport. In this thesis, the vibration dose value (VDV), estimated vibration dose value (eVDV) and root mean square (RMS) were measured to evaluate the effect of vibration. Generally, it is important to note that major parameters to improve animal welfare involve improving the design of loading ramp and handling facilities; avoiding aversive conditions like long transport time, avoiding transport by walking and thus exposing animals to traffic accidents; and avoiding loading animals onto substandard or poorly designed vehicles.

5.2. Modelling of heart rate during loading for transport

The heart rate simulation model developed in Paper I of this thesis described well the dynamic behaviour of heart rate response of cows and heifers during loading for transport. The heart rate rose exponentially from its mean resting value to a peak value about 1.9 times the value at resting, and then declined during a recovery period to about 1.15 times the resting level. The amplitude of the rising part of the heart rate response was not significantly different for heifers and cows. Similarly, the amplitude of the decaying part of the heart rate response for heifers and cows was similar on average. In all cases, because of differences in stress levels, it was observed that the heart rate value did not stay at the peak value; once the peak value was attained, the rate started decaying during the recovery period. Generally, heart rate is a relevant parameter to investigate animal response to physiological, emotional stressors and to describe the level of stress load on the animal. During transport of sheep, heart rate was observed to remain elevated for at least 9 hour (Parrott *et al*., 1998b). Heart rate variability has also been found to be a useful welfare indicator in cattle and other species (van Ravenswaaij *et al*., 1993).

Heart rate is a useful measure of welfare for short-term stressors such as those encountered by animals during handling, loading onto vehicles and certain acute effects during transport. According to Natalie *et al*. (1995), forcing animals to mount the loading ramp after they refuse because of novelty is among the transport process which cause severe stress. Those authors demonstrated this stress by the high heart rate recorded during loading in their study. The extent of animal responses to painful or unpleasant situations varies from one to another and heart rates are higher for cattle loaded on the vehicle for transport. Heart rate variability is a valuable measure of the welfare of animals, with those animals with greater heart rate variability being the most disturbed (van Ravenswaaij *et al*., 1993; Minero *et al*., 2002). Heart rate variability is also a physiological indicator of stress imposed on the animal. According to heart rate measurement results reported by Gebresenbet *et al*. (2012), loading, unloading and confinement in the stunning box are the most stressful processes during transport of animals. The study found that heart rate and loading time were increased significantly with an increase in ramp angle inclination. The slope of the ramp is an important aspect when loading and unloading animals (SCAHAW, 2002). In a study by Chacon *et al*. (2005), the heart rate of animals increased by 165% as they were forced to climb or mount a steep ramp. Steeper ramps cause greater increases in heart rate, up to a maximum level (Van Putten, 1982).

In this thesis, the responses of the heifers and cows studied were determined by continuous observation of behaviour and measurement of heart rate during loading for transport. During loading, some animals refused to mount the ramp and this led to severe stress in those animals. Loading is generally one of the most stressful components of transportation for animals (Trunkfield & Broom, 1990; Waran & Cuddeford, 1995) and heart rate in animals tends to peak during loading (Stewart *et al*., 2003; Stirling *et al*., 2008). This was shown by the maximum heart rate recorded during loading in Paper I of this thesis, which assessed the heart rate variability and its impact during loading animals for transport. The modelling of heart rate was used as a simplified reproduction of the heart, as modelling can be an important contributor in representing complex changes in heart rate. The results in Paper I showed that modelling of heart rate could be used to explain the dynamic response of heart rate during loading processes.

5.3. Vibration levels and resonance frequencies during transport

The amount of discomfort produced by vibration is dependent on the magnitude and frequency of vibration, the standing orientation of the animals, their body posture and the exposure time. During transport, animals are subjected to vibrations in three orthogonal directions and thus the levels of exposure were evaluated in Paper II of this thesis using relevant vibration parameters. In that study, four driving speeds (30, 50, 70 or 90 km/h) and three road types were used. It was found that road conditions ($P<0.0002$) and standing orientation ($P<0.002$) both 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 with a second peak at about 23 Hz in one specific case and the vibration depended on the speed of the vehicle and the road conditions. According to Carlise (1998), higher vibration frequencies of 5 and 10 Hz or 2 m/s^2 induce more physiological stress than lower frequencies on poultry during road transport. Due to transmission of vibration, the upper part of animals' bodies sways in relation to footing. Here, transmissibility of vibrations between the chassis (input) and the floor (output), and the ratio of floor (input) and to cattle (output) were estimated. In general, it was found that vibration was amplified, as the frequency of vibration corresponded to the resonance frequency of the body or the system. Vibration in the moving vehicle is one of the factors considered to be an acute stressor causing physiological and behavioural stress in animals (Stephens & Rader, 1983), and it may have an influence health, comfort and activities and cause motion sickness (BSI, 1987). In the three orthogonal axes, VDV, eVDV and RMS were higher on the chassis than on the vehicle floor and animals. 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, whereas the contact between the floor and the animal is not rigid and it has no vibration reducing system. Moreover, as the upper part of the animal's body sways in relation to the footing, vibration levels on the upper body of the animals could be higher than on the floor. The main reason for amplifying vibration on the animal was found to be the rigid interface between the vehicle and the animal and the lack of damping structure to reduce vibration levels. Furthermore, VDV, eVDV and RMS were found to be higher on cattle than on the floor along the horizontal and lateral directions, but lower in vertical direction.

During vibration measurements on different road conditions and speeds, the highest values measured on animals in the driving direction on gravel roads at speeds of 50 and 70 km/h were 2.23±0.27 and 2.27±0.33 m/s^2, respectively. Peeters *et al*. (2008) reported that during short journeys in pig transport, the lowest VDV values were recorded with a quiet driving style and the highest VDV were recorded with a wild driving style. Those authors also reported that driving style had an effect in the horizontal and lateral directions, with animals experiencing lower horizontal vibrations when facing perpendicular to the driving direction than when facing in the travel direction. In another study, pigs with a body weight between 20 and 25 kg were vibrated in the vertical direction for 2 hour at 2, 4, 8 and 18 Hz, in combination with RMS acceleration magnitudes of 1 or 3 m/s^2 (Perremans *et al*., 2001). The results showed that at the beginning of vibration exposure, the pigs were extremely susceptible to vibrations at lower frequencies (2 and 4 Hz), whereas at the end of vibration exposure the responses were higher at 18 Hz. In this report, the animals were exposed to lower levels of lateral acceleration in the perpendicular direction to the travel direction. 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). It is known that the vibration varies with the suspension type, tyre pressure, shock absorbers, applied speed (constant, speeding up or braking) and road quality (Randall *et al*., 1993; Smith *et al*., 1996). Animals facing vibration in the travel direction are exposed to a greater degree of loss of balance because of speed variations and braking and the vibration intensity can be used to characterise the non-stationary nature of vehicle vibrations (Li & Jansen, 1991; Rouillard & Sek, 2000; Rouillard & Richmond, 2007; Lu *et al*., 2008). In this thesis, the combinations of vehicle speed, road conditions, driving performance and cattle standing orientations tested had significant effects on the level of vibration.

In Paper II of this thesis, an experimental and theoretical investigation was made of vibration exposure in animals during transport. This was assessed in terms of different speeds and road types and the results indicated that road type, exposure time and vehicle speed had impacts on the animals during transport. It was also observed that factors determining animal exposure to vibration included the magnitude (level) of vibration and length of exposure time. The longer the duration of exposure time for the animal, the greater the risk of vibration exposure. The point of entry also had a major influence on how vibration was transmitted through the animal body. Overall, transport of animals by vehicle on rough secondary roads and other related stressors caused due to vibration are more stressful for animals. In general, to reduce the transmission of vibration from vehicle floor to the loaded animal, improving vehicle suspension, improving the performance of the driver in terms of controlling the speed and swaying of the vehicle and minimising exposure time of the animals are very important.

5.4. Transport time and animal welfare

Transport and handling activities and transport time have significant effects on animal welfare as a whole, as indicated in this thesis by the results obtained for blood parameters, behaviours and climate conditions inside the vehicle. During transport, cortisol, glucose, lactate and creatine kinase proved to be relevant parameters to describe the stress levels imposed on animals. For example, cortisol concentration was increased by the stress of loading and initial transport, but decreased with increasing transport time. It is an indicator of stress caused by fear and arousal and showed the immediate response of the animals. In winter and summer experiments in pigs, glucose concentrations were highest for 8 h transport and lowest with 12 h transport time. Lactate concentration increased with an increase in transport time during winter. Concentration of creatine kinase increased exponentially as transport time increased from 4 to 8 and to 12 h, with 154 μ mol/L as the maximum value noted at 12 h transport time during summer experiments. In comparison, the control values varied between 115 and 129 μ mol/L for pigs. After shorter journeys, more pigs arrived by standing than after longer journeys, where animals were more fatigued. Grandin (1994) and Knowles (1999) also found that after long journeys, animals tended to lie down for the latter part of their transportation time. The welfare of transported animals decreases as the length and time of transport increases (SCAHAW, 2002). During transportation, animals may lose their balance or fall. Both those events can be associated with the vehicle structure and the loading density (Tarrant *et al*., 1988, 1992; Gallo *et al*., 2001). During transport, the pigs observed in Paper III of this thesis showed many different behavioural patterns depending on loading method, transport time and handling. The number of lying pigs was 60% after 2 hours and 80% after 6 hours and the lying behaviour increased constantly from 4 hour to 8 hour and then up to 12 hour transport time. It is clear that pigs should be given enough space to lie down, especially on long journeys, as is mandatory in the EU regulations on protection of animals during transport. Sitting and lying one on another of animals' behaviour were observed as transport time increased, but at different rates. This lying behaviour of animals is the result of the behaviour they developed on the farm.

During handling and transport, animals are subjected to many different potential stressors such as heat, cold and poor air quality, which affect the welfare and health of animals up to the point of death (Gebresenbet, 2010). When the pressure exerted by the environment on an animal reaches a certain level, new defence mechanisms can be initiated in response to the new conditions and this response mechanism is referred to as the stress response (Gebresenbet *et al.*, 2010). Fazio *et al.* (2005) found that transport stress triggers an increase in thyroid and adrenal function in cattle that is evident after short journeys and continues to increase after long-distance transport. In many studies, cortisol is reported as to be an immediate response or time-dependent hormone, with its secretion increasing in response to physical and psychological stress during the fight or flight response, while at higher levels it contributes to reducing the immune response and increasing blood pressure. According to Grandin (1997), the most common physiological measures of stress are cortisol, beta endorphin and heart rate. Glucose serves as the primary energy source for the brain and a source of energy for cells throughout the body. According to Gebresenbet *et al.* (2012), glucose concentrations increase with transport time in cows, bulls and calves, and transport time has a significant effect on concentration of glucose. In the cows studied in Paper IV of this thesis, concentration of glucose generally increased with an increase in transport time, but in winter and summer the glucose concentration was lowest at 8 hour and highest at 12 hour transport. Glucose concentration in bulls increased in proportion with the increase in transport time. Lactate plays an important role in energy metabolism. The elevation in lactate concentration in bulls was highest during winter at 8 hour transport and during summer at 12 hour transport. The highest lactate concentration in bulls was observed at 12 hour summer transport time. The highest lactate concentration in cows was observed at 4 hour and 12 hour winter transport time and it was significantly higher than the control value. This indicates that transport is a source of stress, even under optimal conditions (Grandin, 2000). Observing appropriate rest periods and limiting the duration of the journey are the best ways to avoid stress turning into distress.

Creatine kinase is a muscle enzyme found in brain, heart and skeletal muscle and elevated concentrations expose animals to poor welfare. The appearance of creatine kinase in blood is generally considered to be an indirect marker of muscle damage and vigorous exercise**.** The continuous increase in creatine kinase with an increase in transport time noted in Paper IV indicated increasing muscular fatigue, which could be attributed to restlessness, loss of balance and fighting during transport. The plasma levels of creatine kinase increase with the length of the journey, but also remain high for several days after transport (Warriss *et al*., 1995; Knowles *et al*., 1999a). In Paper IV, the concentration of creatine kinase in cows and bulls was correlated with transport time during both seasons (winter and summer). The high concentrations recorded during the experiments were presumably related to muscular fatigue resulting from vigorous exercise (loss of balance, restlessness and change of position, reversal, and aggressiveness, fighting and swaying behaviour) of the animals.

Behaviour is known to be one of the most important traits in animal life. The major indicators of an animal having difficulties in coping with handling and transport are changes in behaviour, which show that some of the situations are aversive or not aversive. In Paper III, behavioural alterations, particularly lying, sitting, rooting, vocalisation, smelling, restlessness, change of position and panting, were correlated with transport time in pigs. These behavioural changes indicate that understanding animal behaviour can be used to improve design of handling systems and hence efficient use of labour in handling livestock (Grandin, 2000). During loading the most common behaviours of the bulls and cows studied in Paper IV were elimination, smelling, vocalisation, aggressiveness and refusal to mount the ramp. Other behavioural indicators of discomfort include attempting to escape, vocalisation, kicking or struggling (Grandin, 1997). Refusing to mount the ramp had the highest scores in both animal categories studied in Paper IV (cows and bulls), reflecting the high stress level that was the consequence of separation and new environment. Behaviour plays a critical role in the adaptability of animals to their new environment. Loss of balance can be attributed to driver behaviours such as braking, stopping and cornering, and falling is less evident at high stocking densities compared with low (Tarrant *et al*., 1989, 1992). Swaying and loss of balance behaviours make a contribution to affecting animal welfare in the form of bruising, injury and fatigue. If loss of balance behaviour occurs continuously and is exaggerated, cattle may fall and may have difficulty in standing up. The extent of behavioural responses to painful or unpleasant conditions varies from one species to another, based on the stressor or pressures on the animal.

In this thesis, high initial relative humidity was shown to contribute to a rapid increase in humidity, together with rising temperature. However, when the initial relative humidity did not exceed 40%, the increase in humidity in the vehicle was gradual. The reason for high initial humidity (>40%) was the high availability of moisture after washing the vehicle and insufficiently ventilated floors and side walls. When the amount of moisture in the atmosphere does not change, there is an inverse relationship between temperature and relative humidity. In cattle transport vehicles, the temperature inside the vehicle is not the main factor influencing the relative humidity. Under natural conditions, animals in the vehicle produce heat from respiration, urination and defecation and the magnitude of this heat increase depends on the number and size of the animals. The exchange of heat between the animals and the vehicle container is a continuous process. When temperature is falling and relative humidity is rising, welfare of animals is impaired not only because of temperature, but also because of the combined effect of temperature and relative humidity.

Transport in general increases the risk of animal exposure to stressors and to environmental influences that are caused by the means, condition, process and duration of transport. The constraints to which animals are subjected during transport include loss of balance, physical injury, swaying and other stressors. Measurements of stress-inducing factors such as injury, bruising, loss of balance, vocalisation, and fighting, restlessness and carcass quality can be used as indicators of welfare during handling and transport. The type of vehicle that carried the animals and the road conditions were found to contribute to create uncomfortable situations in the present work, by throwing the animals around, and ultimately resulted in poor meat quality.

5.5. Transport of Animals by walking

Transportation is a very complex and difficult event for all animal categories, in particular for those animals transported by trekking/walking. During animal transport by walking, the associated activities and processes create severe stress conditions which affect the well-being of animals. Thus transport of animals in this way and their handling during the walk needs good facilities, care and attention to reduce the effect of transport activities that affect animal welfare. Maria *et al*. (2003) reported welfare using a scoring system to evaluate the stress on cattle with special emphasis on loading and unloading. In Paper V of this thesis, the information gathered showed that of the total number of animals transported to Gudar livestock market from feeder markets during the study period, about 7.6% died, about 6.9% were injured and about 2.8% were stolen on the way. In most developing countries, transport systems are generally less advanced, animals are moved over long distances and it is common for substandard vehicles and other procedures to be used.

During transport by walking from Gudar on to Finfinnee city market, of the total number of animals observed about 16% died (including traffic accidents) and 10.7% were injured and the animals were not protected from extreme heat or sunburn and wind chill. A study by Gregory (2008) found that about 34% injuries in cattle transport in Nigeria were caused by the horns of cattle, while in Bangladesh the injuries incurred during animal transport included abrasions, scars, lacerations, swelling, penetration and bleeding. Gregory (2008) also reported that animal welfare concerns such as fatigue, fear and distress, fasting, dehydration and injuries were more common in animals bought through livestock markets in those countries. In Paper V, behavioural stress was observed as soon as the animals entered through Gudar market gates. Some animals showed behavioural changes such as curiosity, ear or tail erection, vocalisation, random jumping, refusal to move forward, running away, fighting with their handlers and with each other, urinating repeatedly, and being aggressive. The length of journey (duration and distance), lack of food, water and rest during transport by walking made the animals exhausted, dehydrated and severely stressed. Some were injured on the way, suffering from painful problems such as lameness, swollen legs, loss of weight and broken horns.

In general, the animal welfare issues observed at Gudar market were caused by poor vehicle design and infrastructure; lack of enforcement of legislation; and inhumane handling of livestock throughout the transportation chain. Animals transported from farm or point of origin to Gudar market and then to Finfinnee market were transported a total of about 369 km (319 km by walking), which took 100 hour (95 hour by walking).

In transport processes, it is important to improve the handling facilities and avoid aversive conditions like long duration transport by walking, exposing animals to traffic accidents, high values of climate parameters, or driving animals on asphalted or gravel roads without food, water and resting time. Gentle handling in well-designed facilities will minimise stress levels, improve efficiency and maintain good meat quality. Rough handling or poorly designed equipment determines both animal welfare and meat quality. Journeys by walking result in poorer welfare than journeys by vehicle, and also result in more welfare problems. As mentioned above, there are a number of animal welfare concerns in developing countries that subject animals to a high incidence of death, sickness and non-ambulation of slaughter animals during transport to the abattoir. A recent study in Ghana indicated that about 7% of cattle supplied to an abattoir were 'downer' (non-ambulatory) animals (Frimpong, 2009). In Paper V, it was observed that during transport by walking, animals died due to car accidents, or were injured or stolen on the way. Hence any journey by walking should be as short in distance and time as possible. It was observed that animals were sometimes forced to walk on asphalted and sharp gravel on the road, which could injure animal hooves during long journeys and affect the welfare of the transported animals. Understanding how transport processes affect farm animals and their treatment can help in avoiding unnecessary long-distance transport of livestock to slaughter and in reduction of welfare problems.

5.6. Ambo abattoir

The abattoir system in developed countries is quite different from that in developing countries. The abattoir system in developed countries provides services that pay attention to meat quality, whereas most of the abattoir systems in developing countries, including Ambo abattoir in Ethiopia, provide services for institutions, butchers, restaurants and hospitals and focus on the quantity of meat required, without due consideration of meat safety and quality. Ambo abattoir is the only small-scale abattoir in Ambo town and is situated near a river used for drinking water and household needs. Animals bought at Gudar market by traders, butcheries, institution and individuals are slaughtered at Ambo abattoir. The activity chain of Ambo abattoir has a structure of different operations such as animal delivery from market to stunning place, slaughter operations from stunning to evisceration, packaging and distribution of meat, delivery of skin to market, and waste disposal. In Ethiopia, cattle are slaughtered without stunning because of traditions and lack of knowledge about modern slaughter techniques or systems. The slaughtering system was very traditional, similar to that used by local farmers, there is no cooling room and no machine operations, and thus the abattoir lacks the basic facilities which a standard abattoir should have. Edwards *et al*. (1979) examined slaughter facilities for tropical countries in urban and rural locations and found that each has advantages and disadvantages. He recommended that the abattoir should be built on firm, gently sloping land away from other buildings, residential areas and factories. He also suggested that the site of the abattoir should be placed well away from town boundaries, including projected town boundaries. Abattoir management provides a service in slaughtering animals. Edwards *et al*. (1979) found that the slaughter of animals in abattoirs in developing countries is often carried out in unsuitable buildings by untrained slaughter men and butchers who are unaware of sanitary principles. Wastes generated by abattoirs are potential environmental quality problems. In Ambo abattoir, the waste from the slaughterhouse, especially liquids, is simply discharged to the river and there is no special waste disposal system. According to Raymond (1977), such improper animal waste disposal can lead to animal diseases being transmitted to humans through contact with animal faeces and can affect water, land or air qualities if proper management practices are not followed. Generally, Ambo abattoir does not fulfil the hygienic requirements to produce safe and wholesome meat. The sanitary conditions on the slaughter slabs and within the abattoir grounds determine the effect on the water quality of the wells used in dressing carcasses and other activities (Adeyemo *et al*., 2002). Improvements are needed in all areas. According to a medical report by Oyedemi (2004), the diseases caused by abattoir activities include pneumonia, diarrhoea, typhoid fever, asthma, wool sorter disease and respiratory and chest disease. These diseases can spread from the abattoir to the neighbourhood via *e.g*. insect or animal vectors or other means of transmission. A growing population and an increasing demand for meat have resulted in increased abattoir-related pollution in developing countries.

The problems observed in Ambo abattoir were poor animal management, lack of basic necessary equipment, lack of trained staff, hygiene problems, environmental pollution and impaired health of residents through the abattoir being located in a residential neighbourhood.

5.7. Remarks

This thesis shows that animal welfare problems in relation to handling, transport, marketing and transport processes are diverse and wide. Aggravating factors include transportation, especially over long distances and time, loading or unloading, vibration, slaughter and pre-slaughter management, vehicle design, facilities with adequate feed and water, the handling of animals by their handlers, the protection given to unhealthy animals by carers, marketing situations and stocking density during transport. In most developing countries, the concept of animal welfare is as not well understood as it is in developed countries. In addition, very limited information is available on animal handling and transport during marketing and at the abattoir. Animal welfare could be compromised during rearing, transport from farm to market and transport from market to abattoir. Transport of animals to and from markets, handling at the abattoir and, in particular, transport by walking affect the welfare of animals. In Ethiopia, during transport by vehicle animals are exposed to poor loading and unloading facilities, solar radiation, tied by ropes to the side of the vehicle and swaying around because of poor road and driving conditions.

In general, the issues that need attention are factors that cause stress to animals, such as social regrouping, climate factors, transport processes and length of transport time, feed and water deprivation. The development of scientific research regarding animal welfare is the key element to implementing good animal welfare practices. Future progress in scientific research and improvement of welfare of animals should be based on assessments of the risk factors and on improving the overall system of animal handling and transport. Animal welfare problems require attention and recognition and also need remarkable solutions, which could contribute greatly to economic success and social development.

6. Conclusions

The welfare of animals during handling and transport was assessed using a range of behavioural, physiological and carcass quality measures. Animal handling, transport activities and transport time had significant effects on pig and cattle welfare. Studies of transport conditions, vibration levels, animal behaviour, stress hormones and pH_{24} values indicated that transport and its associated processes had a negative effect on animal welfare. The dynamic simulation model developed to describe the dynamic performance of heart rate was able to simulate the trajectory of heart rate signals over the entire time window of loading heifers and cows. The simulated data correlated well to the measured data ($R^2 = 0.89 \pm 0.06$). The results showed that the heart rate of the animals increased exponentially from its resting value to a peak value and decreased exponentially from the heart rate maximum during recovery. However, the rate of rise was nearly twice the rate of decay.

In studies on vibration levels and frequencies during transport, the highest vibration values measured on animals were 2.23 ± 0.27 and 2.27 ± 0.33 m/s^2 in the driving direction on gravel roads for driving speeds of 50 and 70 km/ h, respectively. Road conditions and standing orientation influenced actual vibration levels. The most common resonance frequencies of vehicle vibration were 1.3, 5.1, 12.6 and 23 Hz. In the horizontal and lateral directions, VDV, eVDV and RMS were greater on animals than on the vehicle floor. Transmission of vibrations from the vehicle chassis to the floor was damped to a certain degree, but vibration transmission from the floor to the animals was 130% and 158% in the horizontal and lateral directions, respectively, and was amplified in the animal body.

In physiological measurements examining the effect of transport time on the welfare of pigs, cows and bulls, cortisol concentration was significantly elevated during short transport time and decreased with increased transport time. An increase in creatine kinase during transport indicated increasing muscular fatigue. The rate of increase in creatine kinase concentration was higher when transport time increased from 4 to 8 h than from 8 to 12 h for cows (during winter) and bulls (winter and summer). Behaviours that scored high values were positively correlated with transport time. Behaviours like rooting, reversal and vocalisation were observed only during loading, showing that the severity of stress is higher during loading than unloading. During summer, loading also caused thermal stress. Inadequate ventilation after vehicle washing was found to be the main cause of high initial relative humidity.

A study on cattle handling and welfare issues during transport by walking from feeder markets to Gudar livestock market in Ethiopia and on to Finfinnee city market indicated that long journeys had a negative impact on animal welfare. Of the total number of animals transported from farms to Gudar market in the study period, about 7.6% died, 6.9% injured and 2.8% were stolen on the way. During walking from Gudar to Finfinnee, of the total number of animals observed about 16% died and 10.7% were injured. Lameness, injuries to bone and muscle, leg swelling and sickness were widely seen during the journey by walking. Other problems observed were lack of knowledge among farmers and traders; difficulties in getting timely market information; poor market infrastructure; and lack of links between farmers and traders. Slaughter procedures and meat distribution to butcheries and other institutions were performed in unhygienic conditions, compromising meat safety and quality. Overall, animal welfare was compromised because of inappropriate transport processes, lack of facilities in the marketing area and poor abattoir systems in Ethiopia.

Animal transport vehicles must be designed and equipped with the necessary devices to control climate, conditions in the vehicle and the behaviour of the animals. Behavioural parameters, stress hormones, carcass pH value and environmental conditions in combination provide a better picture of animal welfare level during transport. The overall conclusion from the studies of cattle and pig welfare and transport time, based on climate conditions, animal behaviour, stress hormones and final pH values in carcasses, was that an increase from 4 to 8 h transport time had a higher effect on animals' welfare and subsequent meat quality than an increase from 8 to 12 hours. Most of stress hormones and behaviours measured increased slightly or remained at a steady state between 8 and 12 hours of transport.

Recommendation

- During transport by walking, animals must be handled in a safe and suitable way to ensure they are free from hazards and stresses that can cause poor welfare. Animal transport by walking should be avoided during extreme weather conditions and should be limited to short journeys. Transporting animals for a long time without food, water and enough resting time creates severe stress on animals and highly compromises animal welfare.
- Improving vehicle design is important in improving animal welfare and meat quality. Using substandard vehicles for animal transport, as is done in most developing countries, subjects animals to severe stresses like loss of balance, injuries, bruising and suffering. Vehicles for animal transport should include ventilation and use of appropriate loading/unloading facilities is very important and highly advisable.

Future Research

The work in this thesis raised many issues that could be recommended for further studies. These include:

- ❖ Vibration and its consequence of motion during transport affect animal welfare and are harmful for animals that exposed to it. This thesis investigated the vibration levels on vehicle chassis, floor and animal. However, the consequences of vibrations in relation to animal heart rate were not investigated and further research is needed in this area.

- ❖ During transportation of pigs, strong, repeated behavioural changes were observed and the reason for these behavioural changes needs further study.

- ❖ Transporting animals by walking compromises animal welfare. The problems reported in this thesis require further research through continuous measurement and observation during transport in order to devise scientific solutions. That could improve the economic benefits derived from animal resources and improve food security and sustainable development.

References

Adeyemo OK, IO Ayodeji, CO Aika-Raji, (2002).The water quality and sanitary conditions in a major Abattoir (Bodija) in Ibadan, Nigeria. African Journal of Biomedical Research, ISSN: 1119-5096

Boissy A., Manteuffel G., Jensen M. B., Moe R.O., SpruijtB.,Keeling L.J., Winckler C.,Forkman B., Dimitrov I., Langbein J., Bakken M., Veissier I., Aubert A., (2007). Assessment of positive emotions in animals to improve their welfare, Physiology & Behavior 92, 375–397

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

Brian, G., Sims, B.G., Inns, F., O'Neill, D., (2003). Equipment for working animals with emphasis on equids in developing countries, TAWS Workshop Held 24 April 2003, Silsoe Research Institute, UK

Buzdugan, G., Miha` ilescu, E., &Rades, M. (1986). Vibration measurement, Bucharest: MartinusNijhoff Publishers.

BuckhamSporer K R, Weber P S D, Burton J L,EarleyB 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

Carlise AJ., Mitchell MA., Hunter RR., Duggan JA., Randall JM, 1998. Physiological responses of broiler chickens to the vibration experienced during road transportation. British poultry Science, 39,S48-S49

Chacon, G., Garcia-Belenguer, S., Villarroel, M. and Maria, G.A. (2005) Effect of transport stress on physiological responses of male bovines. *Deutsche tierärztliche Wochenschrift – German Veterinary Journal* 112, 465‑469.

(EC) No 1/2005 impact of Regulation on the protection of animals during transport. Willy Baltussen Girma Gebrensbet Kees de Roest April 2011.

Edwards E, Hector OA., Norman GA, Silverside D 1979. Slaughter Facilities for Tropical Conditions: A Guide to the Selection and Costing of Appropriate Systems. London: Tropical Product Institute J Soc Sci, 19(2): 121-127 (2009)

Fazio, E., Medica, P. D., Alberghina, 2005, Effect of long-distance roadtransport on thyroid and adrenal function and haematocrit values inLimousin cattle: influence of body weight decrease, *Veterinary ResearchCommunications* **29**: 713-719

Fraser, A. F., and D. M. Broom, (1990), Farm Animal Behaviour and Welfare (3rd Ed.), BailliereTindall, London.

Fraser, A.F. and Broom, D.M. (1990), *Farm Animal Behaviour and Welfare*. Wallingford: C.A.B.I.

Frimpong,S (2009). “Effect of Pre-Slaughter Handling and Transport on Welfare and Meat Quality of Cattle: A Case Study of Kumasi Abattoir,” Master’s Thesis, Kwame Nkrumah University of Science and Technology, Kumasi,

Gallo, C., Warris, P., Knowles, T., Negron, R., Valdes, A. and Mencarini, I. (2001) Densidades de carga utilizadas para el transporte de matadero en Chile. *Archioos de Medicina Veterinaria* 37(2), 155–159.

Gebremedhim, B. (2007). Heading towards commercialization? The case of live animal marketing in Ethiopia. Addis Abeba, Ethiopia: International Livestock Research Insitution (ILRI).

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 et al., (2005).Effect of transport times on cattle welfare and meat quality. Department of Biometry and Engineering Swedish University of Agricultural Sciences SLU, Sweden.

Gebresenbet et al, (2012). Improving Loading Facilities and Methods to Minimize Stress on Animals during Transport from Farm to Abattoir. Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Gebresenbet et al, (2012). Effect of transport time and handling on physiological response of cattle. Department of Energy and Technology, Swedish University of Agricultural Sciences, box 7032, Uppsala 75007, 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. (1994) Farm animal welfare during handling, transport and slaughter. *Journal of the American Veterinary Medical Association* 204, 372–377.

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.

Grandin, T. (2000) Introduction: management and economic factors of handling and transport. In: Grandin, T. (ed.) *Livestock Handling and Transport*, 2nd edn. CAB International, Wallingford, UK, pp. 1–9.

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

Gregory, N., Fielding, H., von Wenzlawowicz, M., & von Holleben, K. (2010). Time to collapse following slaughter without stunning in cattle. Meat Science, 85, 66–69.

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.

Hall, S.J.G. and Bradshaw, R.H. (1998).Welfare aspects of transport by road of sheep and pigs. *Journal Applied Animal Welfare Science*., 1, 235-254.

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.

Lapworth, J.W. (2004) *Cattle Transport: Loading Strategies for Road Transport*. Department of Primary Industries and Fisheries, Brisbane, Australia.

Luís A. Nunes Amaral, Ary L. Goldberger, Plamen Ch. Ivanovc, H. Eugene Stanley (1999), Modeling heart rate variability by stochastic feedback, Computer Physics Communications 121–122 and 126–128

Li H and Jansen R. (1991), Rouillard V, and Richmond R (2007), Effect of Sampling Parameters on Shock and Vibration Levelsin Truck Transport.

Lu F, et al (2008), Effect of Sampling Parameters on Shock and Vibration Levelsin Truck Transport.

Rouillard and Sek (2000), Analysis and Simulation of Road Profiles. Journal of Transportation Engineering, ISSN: 0733-947X eISSN: 1943-5436. Volume 122.

María, G., Villaroel, M., Gebresenbet, G. (2003). Scoring system for evaluating the stress to cattle of commercial loading and unloading. The Veterinary Record, volume

Malena, M., Voslarova, E., Kozak, A., 2007, Comparison of mortality ratesin different categories of pigs and cattle during transport for slaughter, ActaVeterinaria Brno 76: S109-S116154, issue 26, 818-821

Minka N.S., Ayo J.O., (2007). Effects of loading behaviour and road transport stress on traumatic injuries in cattle transported by road during the hot-dry season, College of Agriculture and Animal Science, P. M. B. 2134, Ahmadu Bello University, Mando-Kaduna, Department of

Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria, Livestock Science 107, 91–95

Minero, M., et al. (2002). Heart rate variability in dairy cattle: measurement andtime domain analysis. *Vet. Rec.,* in press.Moberg, G.P. and Mench, J.A. (2000) *TheBiology of Animal Stress.*Wallingford, C.A.B.I.

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. 147, S. 455-462*

Natalie K. Waran and Derek Cuddeford (1995)._Effect of loading and transport on the heart rate and behaviour of horses. Applied Animal Behaviour Science 43 (1995) 71 – 81

Oyedemi DTA 2000.The Impact of Abattoir Location and Management on surrounding residents in Ibadan, Nigeria. M. Tech. Thesis (unpublished), LAUTECH, Ogbomoso

Parrott, R.F., Hall, S.J.G., Lloyd, D.M., Goode, J.A. and Broom, D.M. (1998b) Effects of a maximum permissible journey time (31 h) on physiological responses of fleeced and shorn sheep to transport, with observations on behaviour during a short (1 h) rest-stop. *Animal Science* 66, 197‑207.

Peeters E.Deprez K. Beckers F. Baerdemaeker J.De. Aubert AE. Geers R (2008). Effect of driver and driving style on the stress response of pigs during a short journey by trailer. Animal welfare 2008, 17:189 – 196, ISSN 62 – 7286.

Perremans S. Randall J M., Allegaert L., Stiles M.A., Rombouts G., Geers R., (1998). Influence of Vertical Vibration on Heart Rate of Pigs.Journal of animal science.Laboratory of Agricultural Buildings Research, Catholic University Leuven, KardinaalMercierlaan 92, B.-3001 Heverlee, Belgium

Perremans S., J. M. Randall J.M., Rombouts G., Decuypere E., Geers R. (2001). Effect of whole-body vibration in the vertical axis on cortisol and adrenocorticotropic hormone levels in piglets. J ANIM SCI 2001, 79:975 - 981

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.

Ravenswaaij, C.M.A. van, Kolléè, L.A.A., Hopman, J.C.W., Stoelinga, G.B.A., andvan Geijn, H. (1993). Heart rate variability.*Annals of Internal Medicine,* 118, 437-435.

Raymond C L (1977). *Pollution Control for Agriculture.*New York: Academic Press Inc.

Sainsbury, D. and Sainsbury, P. (1988) Livestock Health and Housing. Bailliere Tindall, London. SCAHAW (Scientific Committee on Animal Health and Animal Welfare) (2002) Report on the Welfare of

Animals During Transport. Health and Consumer Directorate-General, Brussels, Belgium.

Saymore P. Ndou, VosterMuchenje and Michael Chimonyo (2011), Animal welfare in multipurpose cattle production Systems and its implications on beef quality. African Journal of Biotechnology Vol. 10(7), pp. 1049-1064, 14 February, 2011 ISSN 1684–5315 © 2011 Academic Journals, Review

SCAHAW (Scientific committee on animal health and animal welfare) (2002) The Welfare of Animals During Transport (Detail for Horses, Pigs, Sheep and Cattle). European Commission, Brussels, Belgium.Available at: http://ec.europa.eu/comm/food/fs/sc/scah/out71_en.pdf

Solomon A., Workalemahu A., Jabbar M.A., Ahmed M.M., Hurissa B., (2003).Livestock marketing in Ethiopia: A review of structure, performance and development initiatives, Socio-economics and Policy Research Working Paper 52.

Stephens and Rader, 1983, Effects of vibration, noise and restraint on heart rate, blood pressure and renal blood flow in the pig. Journal of the Royal Society of Medicine.J R Soc Med,v.76(10); 1983 Oct.

Stephanes and Rader., (1990). The effects of restraint, handling, simulated and real transport in the pig 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.

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.

Tarrant, P.V., Kenny, F.J., Harrington, D. and Murphy, M. (1992) Long distance transportation of steers to slaughter: effect of stocking density on physiology, behaviour and carcass quality. *Livestock*

Production Science 30, 223–238.

Tarrant, P.V., Kenny, F.J. and Harrington, D. (1988) The effect of stocking density during four hour transport to slaughter on behaviour, blood constituents and carcass bruising in Friesian steers. *Meat Science* 24, 209–222.

Tarrant V., Grandin T., (2000). Cattle transport. In Grandin T (ed.) Livestock handling and transport 2nd edn. CAB International, Walling ford. UK.

Tarrant et al.(1989, 1992) Long Distance Transport and Welfare of Farm Animals. https://books.google.se/books?isbn=1845934032

Tegegne A., Gebremedhin B., Hoekstra D., (2006).Input Supply System and Services for Market-oriented Livestock Production in Ethiopia, IPMS Project, ILRI, Addis Ababa, Ethiopia

Warriss, P.D., Brown S.N., Knowles T.G., Kestin S.C., Edwards J.E., S.K. Dolan S.K., Phillips A.J., (1995). The effects on cattle of transport by road for up to fifteen hours. *Vet. Rec. 136, 319-323*

Trunkfield HR, Broom DM (1990). The welfare of calves during handling and transport. Applied Animal Behaviour Science 28:135-152

Virgilio V.Di., Barbierit R, Mahrdi L., Stmno S. and Cemtto S. (1997). A multivariate time-variant AR method for the analysis of heart rate and arterial blood pressure Department of Information and System Sciences, University 'La Sapienza', Rome, Italy PII: S13504533(96)000586

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

Wikner,I.,Gebresenbet, G., Tolo, E. (2003). Dynamic performances of cattle transporting vehicle on Scandinavian roads and behavioural response of animals.German Veterinary Journal, volume 110, 100-104

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

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