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Effect of Transport Times on Welfare of Pigs

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Abstract: The aim of the study was to investigate effect of transport times of up to 12 h on pigs' welfare. An observation box was located on the 3rd floor of the vehicle. Device to measure temperature, humidity and video camera to monitor pigs' behaviors were fitted in the box. Eighteen measurements were performed during two seasons for 4, 8, and 12 h of transport time with three replications. Meat samples were taken from longissimus dorsi (LD) and the carcases were chilled for 24 h at +4 °C for pH determination. To determine cortisol, glucose, lactate, and creatine kinase concentration levels, blood samples were collected before and after transport from 90 pigs and from 20 controls that were not transported for control purposes. To evaluate behavioural alterations, frequency of events and durations were considered. Highest pH_{24} of 5.99 ± 0.29 occurred during summer at 12 h transport time. Cortisol concentrations elevated during short and decreased with an increase of transport time (P < 0.001). Highest and lowest glucose concentrations for winter and summer were at 8 and 12 h transport time, respectively (P < 0.01). Concentrations of lactate and creatine kinase positively correlated with transport time (P < 0.002). Lying, sitting, rooting and vocalization behaviours correlated with transport time (P < 0.009). Certain THI values during summer surpassed normal (≥ 74) level during loading. The pH_{24} value correlated with transport time during summer. Lactate and creatine kinase concentrations increased with an increase in transport time but for Glucose, highest value was at 8 h transport time.

Key words: Animal transport, loading, behaviour, pigs' welfare, stress.

1. Introduction

Transport of pigs occurs on large scale both in Sweden and within EU countries. 3 million (in Sweden), and in EU 255 million pigs were transported and slaughtered in 2008 [1]. In Sweden about 1.9 million pigs were transported to SCAN AB abattoirs in the year [2]. In general, the number of abattoirs has been decreasing and may continue decreasing in the future. As a result transport time and distance have increased and this may compromise pigs' welfare and subsequent meat quality. Transport is generally an exceptionally stressful episode in the life of animals and compromises their welfare [3, 4]. During handling and transport, animals are subjected to various stress inducing activities, and loading is known to be the most stressful phase of transport [5, 6]. In an assessment and hazard characterization, the risk factors were categorized into: vehicle design and performance, transport management, transport duration, loading and unloading methods and facilities and consequences of animal transport [7]. It may not be always that animals adapt to the new environment during transport. The welfare of an animal is its state as regards to its attempt to cope with new environment and includes both the extent of failure to cope and the ease or difficulty in coping [8]. Health is an important part of welfare whilst feelings- such as pain, fear and various forms of pleasure are components of the mechanisms for attempting to cope and so should be evaluated where possible in welfare assessment [9, 10].

When pigs are transported to slaughterhouse the effect of journey on their welfare could be assessed by various stress indicators. Response to a stressor can include anatomical, physiological and/or behavioural changes [11]. Although normal values of various responses differ not only among stocks within classes

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of the livestock where as patterns of responses were similar [11]. In transport studies, cortisol, glucose, lactate and creatine are relevant parameters to describe the stress levels imposed on animals [12].

Bradshaw [5] observed an initial peak in plasma cortisol levels following loading but cortisol levels in transported pigs still remained higher compared to non transported pigs up to 5 h into the journey which suggests that transport itself was stressful. Behavioural parameters that are often used for behavioural assessment of stress response were vocalisation; attempts to run away or just stop moving forward [13]. High pitched vocalisation has been associated with fear response of pigs [14, 15]. Pigs that were mixed during transport had shown to have increased activity, fighting and had higher cortisol levels than pigs that were not mixed [5].

It was reported earlier [16] that, for short trips of fewer than 3 h during moderate weather additional space provided no profits. But on longer trips, more space was required so that the pigs could have space to lie down without being on the top of each other. Physical stress associated fatigue are likely to be higher if pigs stand rather than lie down during journey [3]. Open mouth breathing (dyspnea) is a behavioural sign of acute exercise challenge and reflects increased oxygen demand of exercise in normally sedentary pig [17]. Scoring system was employed to evaluate the stress on cattle, with special emphasis on loading and unloading [18]. Short-term stress immediately before slaughter, for example, can increase the likelihood of PSE development, while stress of a longer duration, caused by long distance transportation, causes DFD development [19].

Animal's thermal indices are related to the ability of an animal to cope with environmental stressors (within limits) by adjusting physiologically, behaviourally, and immunologically to minimize adverse effect [20]. Swine tend to maintain their homoeothermic mostly by reducing heat production at high temperatures in connection with reduced feed intake and physical activity [21-23]. Thermal micro-environmental within the loading compartment poses the greatest threat to animals' welfare and well being. Unlike other stressors that may compromise the welfare, thermal effect can under extreme cases result in mortality [24, 25]. The effects of transport have been the subject of detailed studies to evaluate and identify factors affecting the welfare of animals during and after transport [26].

The main objective of the current paper was to investigate the effect of transport times of up to 12 h on the welfare of pigs when transported from farm to abattoir under conventional conditions in relation to thermal stress, stress hormones, behavioural alterations and pH values.

2. Materials and Methods

2.1 Pigs

In total, 2,753 pigs were transported from various farms to the abattoir and out of these 216 were transported in the observation box and behavioural study was made on them. However, blood samples were collected from 90 pigs for field experiment, and blood samples were also collected for control purposes from 20 pigs that were not transit. The age of the pigs was six months and their average weight was 100 kg.

2.2 Space Allowance

The dimensions of the loading compartments of the vehicle where the experimental pigs were placed were 2.68 m (length) and 2.45 m (width). The mean space allowance was 0.55 m^2 pig^{-1}. The standard space allowance according to EU regulation of 2005 is 0.51 m^2 pig^{-1} weighing up to 100 kg [27].

2.3 Vehicle

A conventional pigs' transport vehicle owned and operated by commercial company was used for the experiment. It was a 3-deck vehicle that equipped with an adjustable loading ramp. The vehicle was a 13.6 m long, tri-axled, air-suspended semi-trailer with a load capacity of 280 pigs. When pigs were in transit, provision of ventilation occurred through apertures positioned along the length of the vehicle body. The observation box was located on the third floor and fitted with instruments to measure temperature, relative humidity and a video camera for monitoring pigs' behaviour.

2.4 Parameters

2.4.1 Stress Inducing Parameters

To evaluate the welfare of pigs during transport, stress-inducing factors associated with pre-loading, loading, transport and unloading were considered. The parameters and factors included were:

- Transport time
- Loading and unloading conditions
- Space allowance
- Total number of pigs in the vehicle.
- Temperature
- Relative humidity
- Thermal humidity index (THI)
- Number of stops
- Loading and unloading times

In addition to the above parameters, specific events such as injury, bleeding and death were documented. Transport time was the only parameter that varied intentionally. Loading and unloading conditions, total number of animals in the vehicle, number stops, and loading and unloading times were documented during each trip.

A Cargolog FAT 90V2 battery-powered electronic logger system was used to measure temperature and relative humidity [28]. The system consisted primarily of two parts: portable, battery-operated recording and communication units. A PC interface unit was used to communicate with the recording unit, download parameters, initiate sampling sequences and download the collected data after recording periods. Measurement of temperature and relative humidity were performed simultaneously and continuously throughout transport time and the temperature-humidity index (THI) was calculated. THI is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. It was calculated to determine the level of thermal stress. It has been developed as a weather safety index to monitor and reduce heat stress related losses. Different animal species have different sensitivities to ambient temperature and the amount of moisture in the air [29]. Thermal-humidity index (THI) was introduced [30, 31] to evaluate the combined impact of temperature and humidity expressed as:

$$THI = 0.8\ t_{ab} + RH\ (t_{ab}-14.4) + 46.4 \qquad (1)$$

Where t_{ab} is dry bulb air temperature (^{o}C) and RH is relative humidity in decimal form.

The thermal humidity index has been used to describe categories of heat stress associated with hot weather conditions. Four categories (thresholds) of THI values have been suggested to provide guidelines for safe transportation of animals. In the current work, the threshold given in Table 1 was used to evaluate the performance of environmental conditions in the vehicle.

2.4.2 Stress Response Parameters

The response parameters included were stress hormones, animal behaviour and meat quality in terms of pH.

2.4.2.1 Blood Parameters and Sample Collection

Cortisol, creatine kinase, lactate and glucose were parameters considered and samples were taken before transport at the farm and after unloading at the abattoir. Blood samples were taken from a total of 90 pigs for the determination of concentration levels of cortisol, creatine kinase, glucose and lactate. Every animal was bled twice, at the farm before the start of transportation and immediately after transportation and subsequent stunning at the abattoir. Blood samples were taken from jugular vein. At least 3 mL of whole blood were obtained using BD vacationer systems (Beliver Industrial Estate, Plymouth, UK) for

Table 1 Recommended THI thresholds for farm animals [32].

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

serum separation and lithium heparin (LH) vacuettes for plasma separation. Collected blood was centrifuged for at least 10 min at 2,000 rpm at room temperature using an Eichemeyer centrifuge. Separated cells and plasma were removed using Pasteur pipettes, placed in 1.5 mL micro tubes and stored at -20 °C until analysis.

In addition, control blood samples were taken from 20 pigs (from the same farm) that were not transported, using the same technique. These controls were used then to:

- compare with cortisol, creatine kinase, glucose and lactate obtained from transported animals, and
- determine variations in cortisol, creatine kinase, and lactate and glucose concentration depending on time of day.

Blood samples for control purposes were collected four times a day (06:00, 10:00, 21:00, and 23:00) to study daily variations in cortisol concentrations. These times were chosen because the samples for the field experiment were also taken at these times.

2.2.4.2 Blood Analysis

The cortisol values in transported and control pigs, were measured using radioimmunoassay Coat-A-Count cortisol kits (Catalog number-TKCO5, Siemens Medical Solution Diagnostics, Los Anglos, CA, USA). At the laboratory, to test the performance of the immulite apparatus, it was calibrated once per month or every time the reagent was changed and it was checked on a daily basis. Serum glucose was analyzed using an automatic Konelab analyser (Thermo Clinical Labsystems Oy, Vantaa, Finland) according to the manufacturer's instructions. Calibration of the Konelab analyser was carried out once per month and checked on daily basis. Lactate levels were measured using a GM7 Analox analyser (Analox Instruments LTD, London, UK). A 7 μL plasma sample was used and calibration was carried out with aqueous 8.0 mL L^{-1} (72.1 mg 100 mL^{-1}) lactate standard. At the laboratory, the Analux analyser was calibrated and tested after analysing every 10 samples. Creatine kinase was analysed using an automatic Konelab analyser (Thermo Clinical Labsystems Oy, Vantaa, Finland). The Konelab analyser was calibrated every 14 days and checked on a daily basis.

2.4.2.3 pH Measurement

Meat samples were taken from the longissimus dorsi (LD). The carcases were chilled for 24 h at +4 °C. During that time, temperature and pH decrease were measured in LD between the 12th and 13th rib immediately after slaughter, and at 5, 12, 18 and 24 h post-mortem.

2.4.2.4 Animal Behaviour

Pigs behaviours were continuously observed and documented at farms (during blood sampling and loading), during transport (in the vehicle) and on unloading at the abattoir by visual observation, portable and fixed video cameras. To evaluate behavioural alterations in response to handling and transport activities, the most common observed behaviours were selected and definitions were given for all selected behaviours based on literature and experiences (Table 2).

2.4.2.5 Final Behavioural Quantification

The most common methods used when studying behaviour alterations were either determining the percentage of animals involved in specific events, or determining the frequency of events. In the current study, the duration of events was also included in a more accurate approach to demonstrate the severity of each factor that caused behavioural alterations. For determination of frequency, occurrence of events and total number of animals in the observation box were used. Occurrence and duration of events were determined from video recordings and documentations

Table 2 Definition of most observed behaviours.

Behaviour	Definition
Ear erecting (Er)	The animal stands and erects up its ears to focus on a particular area or object
Fighting (Ft)	The animal strives to secure more space by attacking other animals
Jumping one on the other (Jn)	When an animal jumps on another animal
Loss of balance (Ls)	The animal has difficulties in holding its natural or walking position and tends to fall down
Lying one on another (Ln)	When any part of the body other than hoves rests on another animal's body
Lying (Ly)	Any part of the body other than the hooves touches the ground or floor voluntarily
Panting (Pt)	Breathing rapidly in short intervals
Rooting (Rt)	When a pig smells and digs with its snout
Smelling (Sm)	The animal bends down to the ground or floor in combination with deep, fast and interrupted breaths to feel the new environment
Sitting (St)	The back of a pig touches the ground, in the mean time supported by forelegs
Restlessness & change of position (Rc)	The animal is constantly in motion and attempts to find convenient standing orientation
Reversal (Rv)	The animal changes direction to move against the animals flow
Vocalization (Vc)	Behavioural response expressing discomfort through frequent, high and constant sound

of observation made during the field work. Therefore, the final quantified behaviour was expressed as the product of frequency of events and duration of events.

$$Frequency = \frac{A}{B} \quad (2)$$

$$Behaviour = Frequency \times t \quad (3)$$

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

2.5 Experimental Design

The study was carried out in a farm-transport-abattoir system. The field measurements were made during two seasons, which included: three transport times (4, 8, and 12 h) and three replications. On each trip blood samples were taken from 5 pigs that were transported from 11 farms to SCAN AB abattoir located in Skara. The journeys were planned in such a way that arrival time was always 09:00 local time. Field measurements commenced with blood sampling at the farm, followed by transport and completed by taking blood samples at the abattoir. Soon after blood collecting, centrifugation occurred at the sampling spots. During loading and unloading, there were constant visual observations assisted by hand held camera. Besides length of loading, unloading times and the slope of loading ramps were documented. Inside the vehicle, behavioural observations were recorded using video camera. Visual and videos data were later used to analyse the behaviours of pigs.

2.6 Data Analysis

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

3. Results and Discussion

3.1 General

Although the selected transport times of 4, 8 and 12 h were supposed to be exact, time deviations arose due to variations in loading and unloading times. The standard deviations for the three classes of transport times were 4 ± 0.51, 8 ± 0.22 and 12 ± 0.15 h, respectively. Average loading and unloading times during winter were 0.65 and 0.16 min pig^{-1}, respectively. However, during summer loading and unloading times were 0.35 and 0.12 min pig^{-1}, respectively.

Table 3 Summary of pH values for different seasons and transport times.

Season	Transport time (h)	pH_0	pH_5	pH_{18}	pH_{24}
Summer	4	6.54 ± 0.32	6.15 ± 0.25	5.67 ± 0.21	5.65 ± 0.17
	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
Winter	4	6.50 ± 0.08	6.49 ± 0.13	5.46 ± 0.12	5.58 ± 0.14
	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

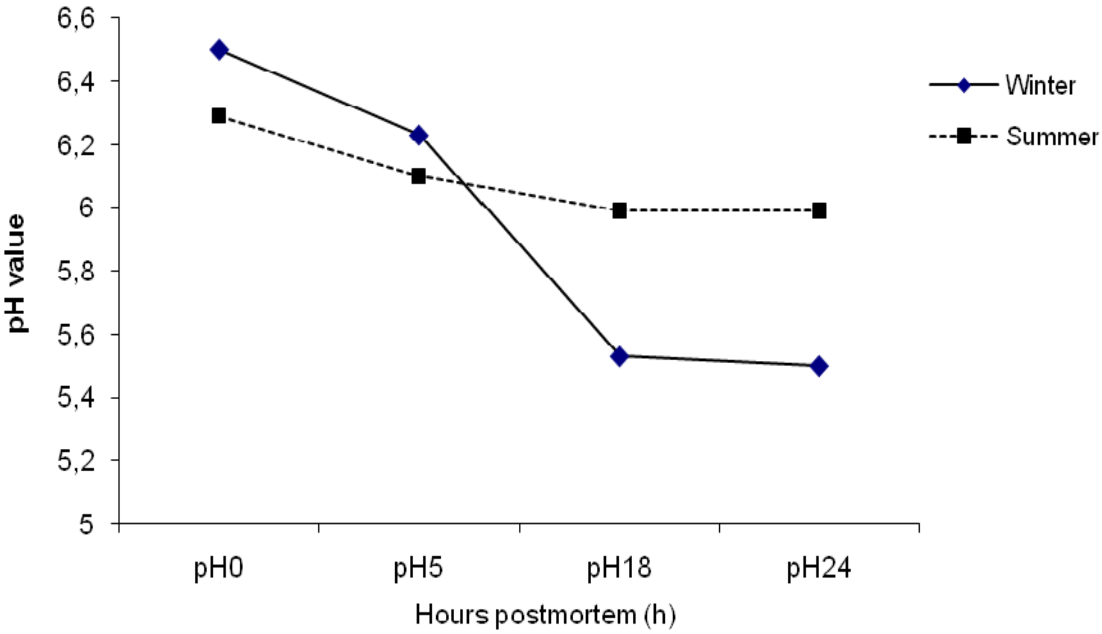

Fig. 1 pH values in pig carcases during winter and summer after 12 h transport.

3.2 pH measurement

A summary of the mean pH values are presented in Table 3. The highest pH24 values for summer and winter were 5.99 ± 0.29 and 5.58 ± 0.14, respectively. Fig. 1 illustrates seasonal effect on pH values at pH0, pH5, pH18 and final pH post-mortem h. During winter measurements, the values of pH0 and ultimate pH ranged from 6.5 to 5.5, where as summer pH values varied between 6.29 and 5.99. The ultimate pH values for the 4, 8 and 12 h of transport times are illustrated in Fig. 2, where each measurement consisted of 3 replications. The lowest (5.5) and highest (6.1) pH values were at 4 and 12 h transport time respectively, besides the lowest (5.5-5.79) and the highest (5.88-6.1) pH ranges were noted at the same transport h. pH24 values during summer correlated with transport time (R^2 = 0.69) .

3.3 Blood Parameters

3.3.1 Control Samples

The values obtained from the control animals were used when performing statistical analysis of the samples gathered during the field experiment. In the control pigs, the highest concentration of cortisol was noted at 06:00 and the lowest at 23:00 h (Fig. 3). In the study the concentration level of glucose ranges from 3.5 to 6.6 mmol L^{-1} and lactate concentration varied between 4.2 and 7.3 mmol L^{-1}. Control values of creatine kinase ranged from 115 to 129 µmol L^{-1} [33].

3.3.2 Transported Pigs

The concentration levels of cortisol, lactate, creatine kinase and glucose for all transport times during the two seasons are summarised in Figs. 4-7. To determine the effect of transport time, the difference between values before and after transport

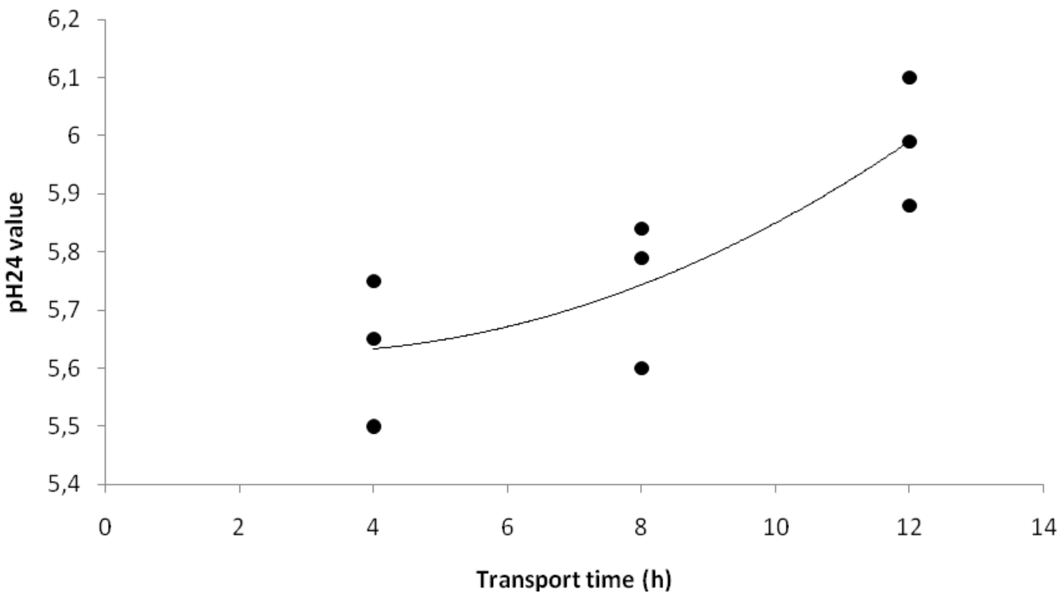

Fig. 2 Effect of transport time on pH24 value in pig carcases during summer, with correlation coefficient, R^2 = 0.69.

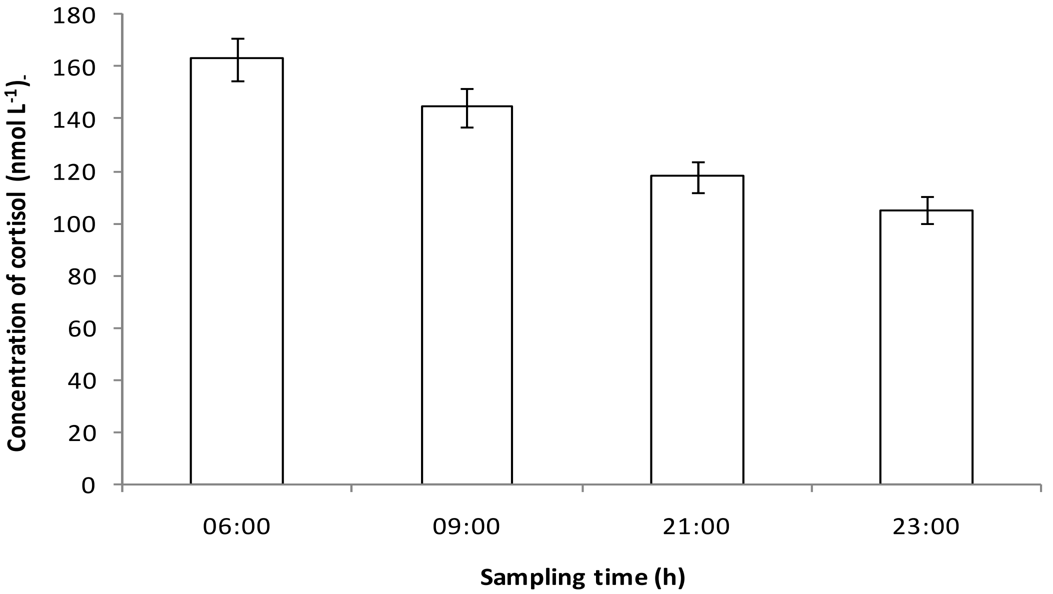

Fig. 3 Variations of cortisol concentration throughout the day in non transit (controls) pigs.

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

The effect of transport time on concentration level of blood glucose was significant (P < 0.01). Glucose concentration increased from short to medium duration transport and decreased thereafter (Fig. 5). Therefore it was highest at 8 and lowest at 12 h transport time for winter and summer experiments. During 8 h transport time, the maximum concentration noted was 20.46 mmol L^{-1}, and that was 3 fold more than the reference value. Concentration levels of lactate (winter) in the blood increased (4.7-6.2 mmol L^{-1}) with an increase in transport time and (Fig. 6) and positively correlated (R^2 = 1) (P < 0.002).

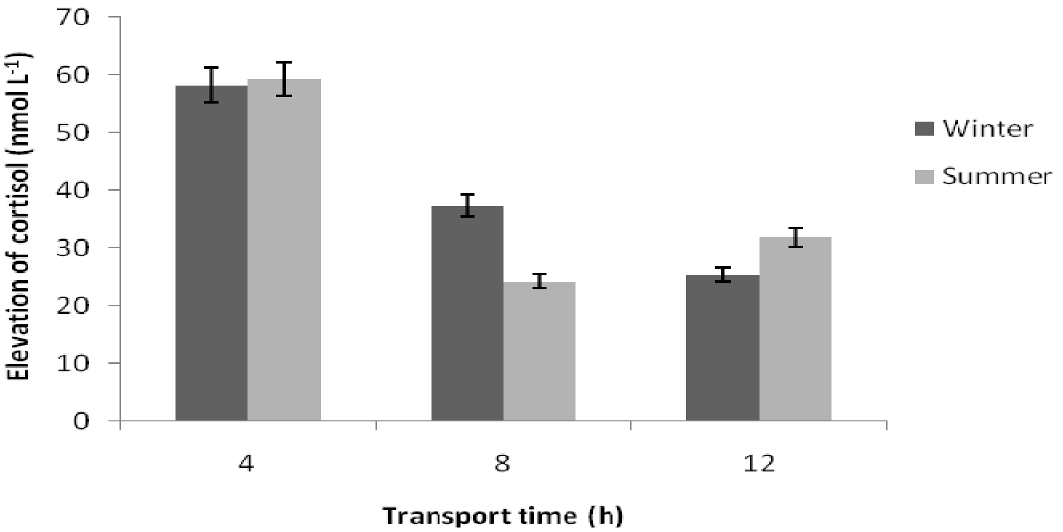

Fig. 4 Elevation of cortisol concentration during winter and summer for different transport times.

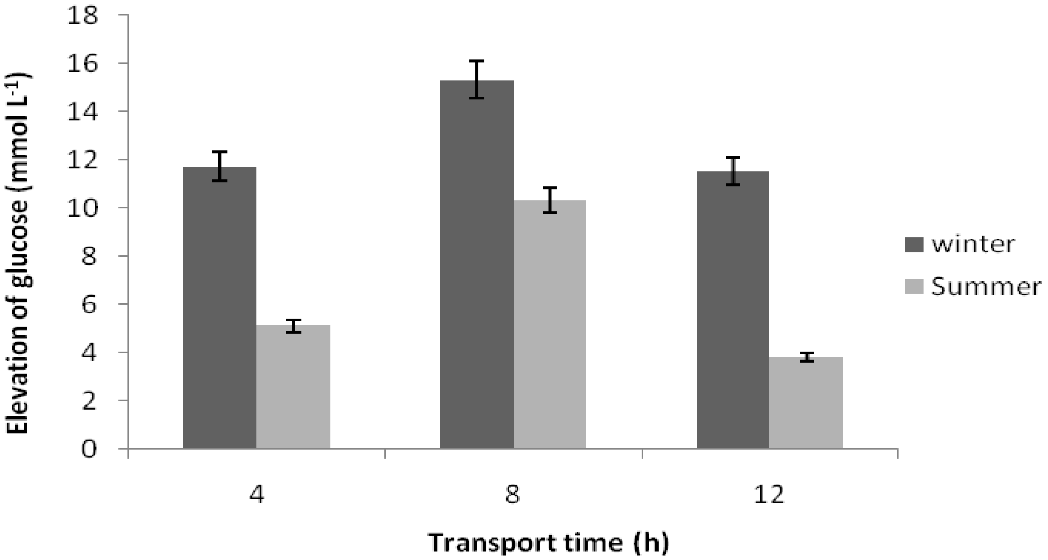

Fig. 5 Glucose concentration level during winter and summer for different transport times.

The elevation in concentration levels of creatine kinase ranged from 0.4 to 25.4 (winter) and 2.5-31 (summer) µmol L^{-1} and thus positively correlated with transport time ($P < 0.002$). The relationship between concentration level and transport time was exponential ($R^2 = 0.99$) for both seasons. The rate of increase in creatine kinase concentration from 4 h to 8 h was lower than from 8 to 12 h transport time (Fig. 7). For summer season and 12 h transport time, the maximum value after transport was 154 µmol L^{-1}, and exceeded the reference value of 129 µmol L^{-1}. In literature, the reference value given for pigs was 30-130 µmol L^{-1} [33].

3.4 Behaviour Observations and Quantification

Behaviours of pigs were quantified as described in section 3 and a summary of the results is presented in Tables 4 and 5. Table 4 presents cumulative frequencies and durations of occurrence representing summer and winter, while Table 5 presents frequencies and duration of occurrence within three intervals (0 to 4, 4 to 8 and 8 to 12 h) of the 12 h transport time to determine alterations in behaviour within the aforementioned intervals.

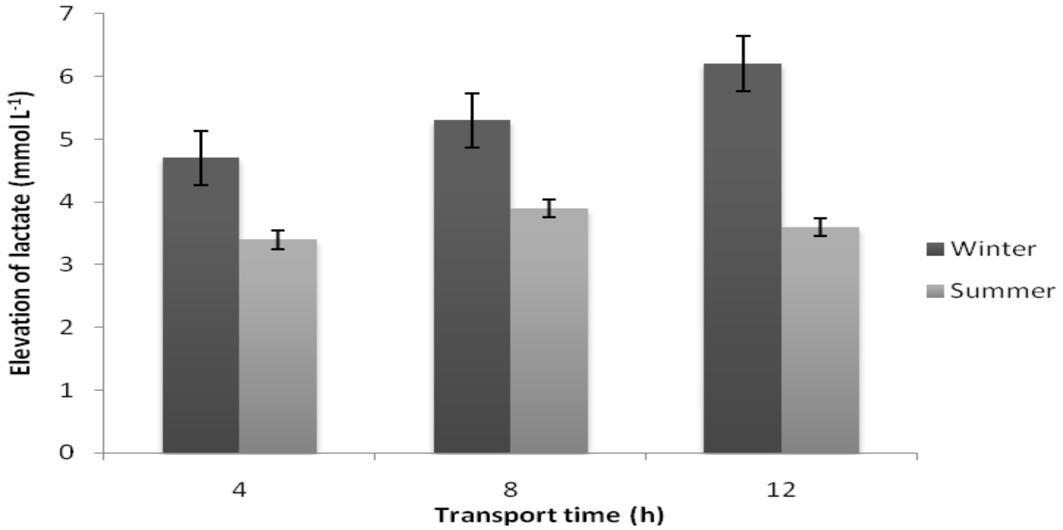

Fig. 6 Lactate concentration during winter and summer for 4, 8 and 12 h transport time.

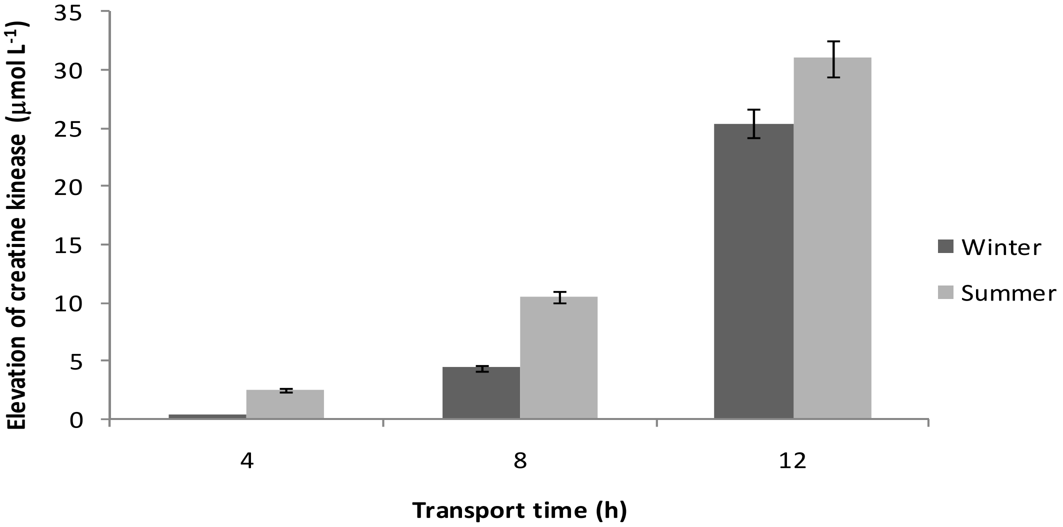

Fig. 7 Creatine kinase concentration level for winter and summer increased exponentially with transport time, R^2 = 0.99.

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

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

Table 5 Observed behaviours within 4 h intervals of the 12 h transport time.

Behaviour	Transport time (h)					
	0-4		4-8		8-12	
	Freq	Freq × time	Freq	Freq × time	Freq	Freq × time
Ft	1.12	0.17	0.3	0.18	0.2	0.1
Jn	0.02	0.01	0.01	0.03	0.02	0.01
Ln	0.02	0.14	0.05	0.19	0.07	0.13
Ls	0.27	0.32	0.32	0.35	0.27	0.31
Ly	1.8	33.13	2.10	403.80	4.43	614.57
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	7.40	0.77	5.25	0.52	3.02
Vc	2.2	3.81	1.8	2.73	1.1	2.52

Lying, sitting, rooting, vocalization, restless and change of position, smelling, panting, loss of balance and fighting were significant and positively correlated with transport time ($P < 0.009$).

When 12 h transport time was subdivided into 3 equal intervals (Table 5), values for lying behaviour increased (33.13; 430.80 and 614.57) as the interval increased from 0-4; 4-8 to 8-12 h respectively.

Smelling, rooting and vocalization were highest at 1st and lowest at 3rd intervals. Panting occurred at 4-8 and 8-12 intervals and loss of balance was nearly the same in all intervals. Restlessness and change of position was highest at 2nd interval and lowest at 3rd interval whereas fighting showed higher values at 0-4 and 4-8 h interval.

Lying behaviour in relation to transport time showed interesting results. During all experiments, about 50% of pigs were lying about two h after the vehicle started moving. About 80% of pigs were lying after 6 h transport time, and the behaviour remained similar until 12 h (Fig. 8). Common behaviours recorded during loading and unloading are shown in Table 6. It represents winter and summer measurements. Unlike other behaviours which occurred at both events,

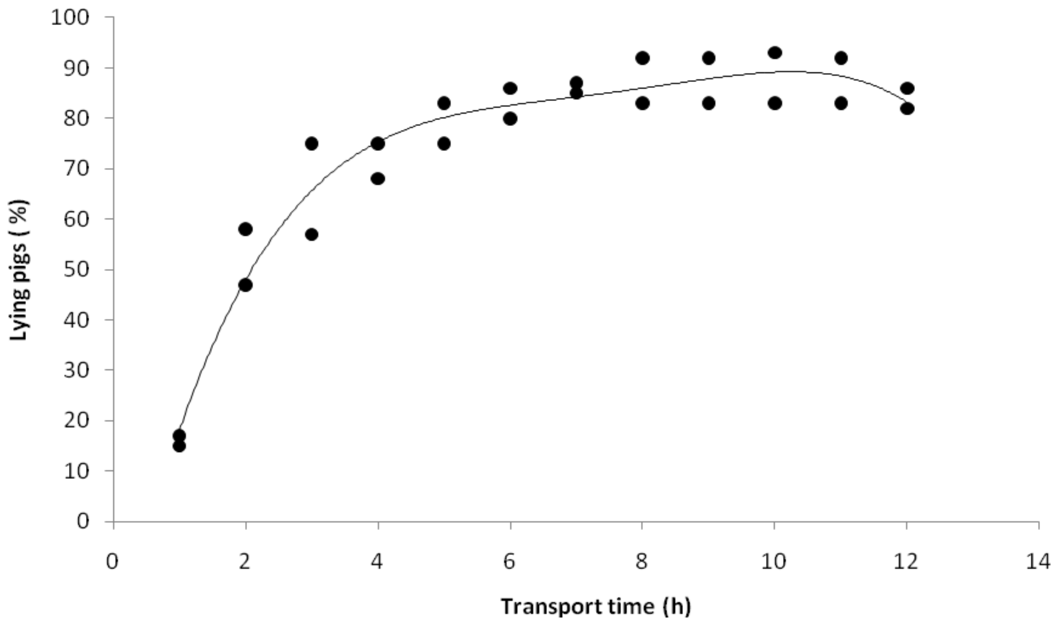

Fig. 8 Proportion of pigs lying in percentage depending on transport time, described with 4th order polynomial function, with correlation coefficient, R^2 = 0.95.

Table 6 Comparison of pigs' behaviour during loading and unloading events.

Behaviour	Loading		Unloading	
	Freq	Freq × Time	Freq	Freq × Time
Er	0.13	0.09	0.1	0.063
Sm	0.31	0.32	0.3	0.34
Rt	0.36	0.4	0.16	0.12
Rv	0.13	0.08	0	0
Vc	0.2	1.0	0.16	0.08

reversal appeared only when loading took place. Even though loading and unloading were independent of transport time, the severity or stress levels were higher during loading.

3.5 Temperature and Relative Humidity

During field measurements the lowest temperature (-2 °C) was recorded in February and the highest (28 °C) in July and the highest value occurred during loading event. In all measurements, whether temperature was rising or falling, relative humidity constantly increased during transport, which was the most common characteristics of temperature-humidity measurements in this study.

Selected temperature and relative humidity data are plotted against transport time. Furthermore, to evaluate heat stress, thermal humidity index (THI) was computed using Eq. (1) and the measured temperature and relative humidity data.

Twelve h winter transport time is illustrated in Fig. 9, with low temperature but high relative humidity at the beginning of loading. The total number of pigs was 135 higher than the average (113) and loading time was 0.73 min pig^{-1} (mean loading time, 0.65 min pig^{-1}). The temperature increased from 4 °C to 17.5 °C, but when loading was completed and the vehicle started moving it went down sharply to 6 °C. The temperature reached its initial level (4 °C) only after 5 h trip.

Initial relative humidity was as high as 94.6% immediate before loading and reached the maximum level as illustrated in Fig. 9. Variation of relative humidity was between 88.1% and 100% (mean: 93.8%), for nearly 7 h transport time. In the remaining about 5 h trip it decreased to a greater range, 68.4%-91.7% (mean: 74.5%).

Fig. 10 shows where both temperature and humidity were high before loading started. Number of pigs in the vehicle was 190, lower than the average (192) and loading time was 0.38 min pig^{-1}, greater than summer average loading time (0.35 min pig^{-1}). At the beginning, temperature was 21.5 °C and it continued rising to 23 °C until loading was completed. Roughly after 1 h trip temperature decreased to 11 °C and thereafter the range was from 11 °C to 20 °C until unloading time. Relative humidity was 85.2% at the beginning of loading and reached the highest level at loading time. After loading it decreased sharply for a short while and again went up to the maximum level. However, it could decrease after about 6 h transport time.

Fig. 11 illustrates the case of low relative humidity and high temperature at the initial case. 198 pigs were loaded in the vehicle and loading time was 0.4 min pig^{-1}. Temperature was 20.5 °C during loading and didn't show further increase. During transport it could decrease to 16 °C and for the whole trip the range was 16 to 21 °C. Initial relative humidity of the vehicle at loading time 58.4%, and the increase of humidity was not rapid and sharp; therefore it reached the maximum level after 70 minutes. After about 3½ h trip, the level of relative humidity started to go down.

Temperature, relative humidity and THI mean values of winter and summer experiments are summarised in Table 7. Relative humidity reached its maximum value both during winter and summer experiments. But the THI mean values of both seasons and for the three transport times (4, 8 and 12 h) were below the threshold value of 74 and the maximum mean value was 65.92. To study the effect of transport time on THI, values of THI within 4-h intervals (0-4, 4-8 and 8-12 h) of 4, 8 and12 h transport time were considered. The maximum, minimum, mean and standard deviations of values of each interval are reported in Table 8. Based on the recommended thresholds (Table 1) THI values were

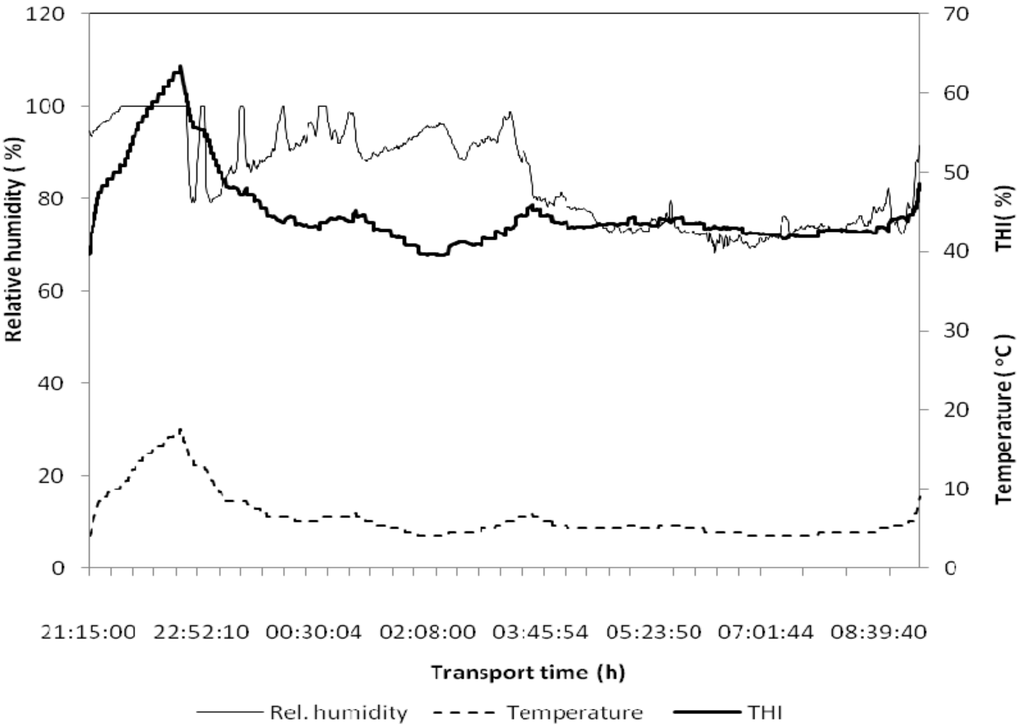

Fig. 9 Temperature, relative humidity and THI variations during 12 h transport time.

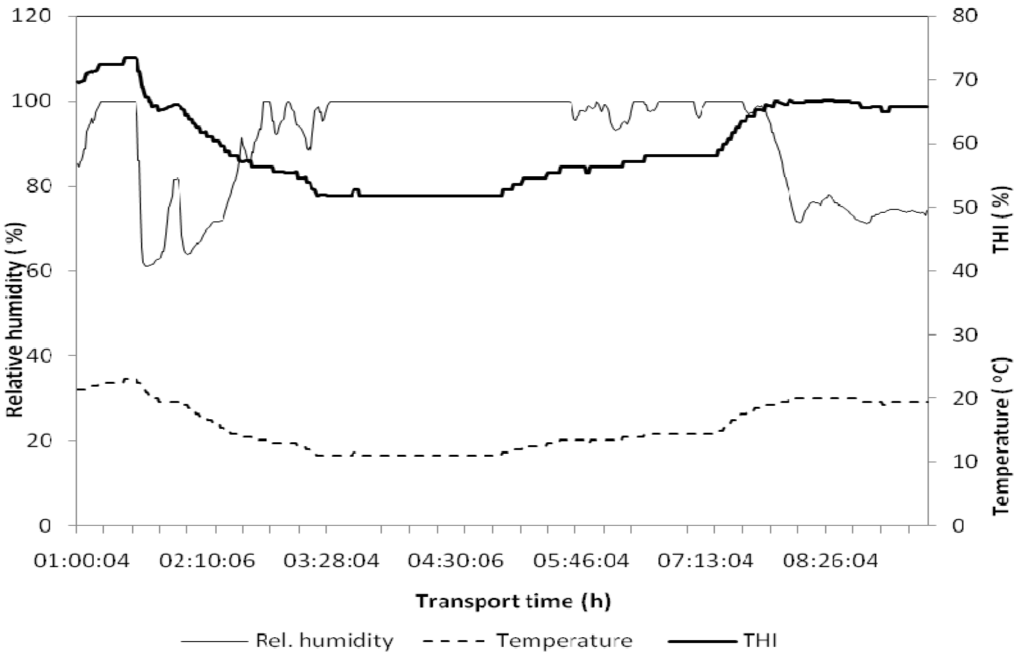

Fig. 10 Temperature, relative humidity and THI variations during 8 h transport time (summer).

grouped according to their level: normal ≤ 74; alert (75-78); danger (79-83) and emergency (≥ 84). The result showed that, during summer season the THI value exceeded the normal value of 74. The maximum value observed was 79.2, which is in the danger zone. As indicated in Table 9, 0-4 h interval, 75.2%, 23.3% and 1.5% of the THI values were in the range of normal, alert and danger categories, respectively and the ranges above the threshold occurred during loading events. Regarding the relationship between THI and transport

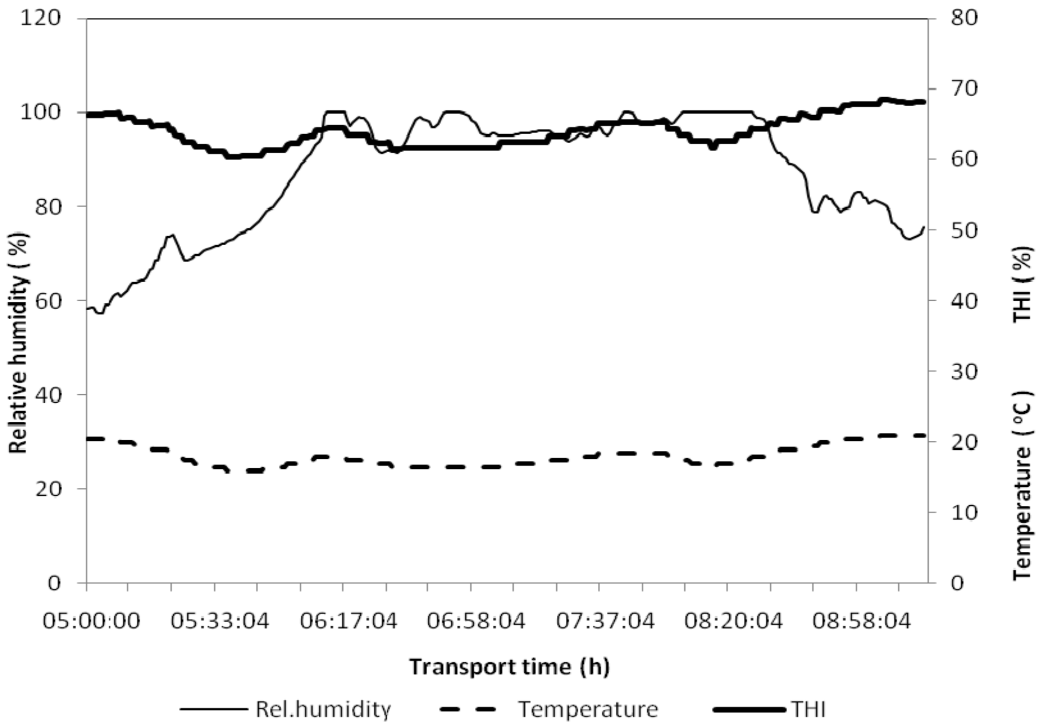

Fig. 11 Temperature, relative humidity and THI variations during 4 h transport (summer).

Table 7 Summary of mean values of temperature, relative humidity and THI in winter and summer.

Season	4 (h)			8 (h)			12 (h)		
	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

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

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

time, seasonal variation had more effect than transport time, as can be observed in Tables 8-9.

3.6 Multivariate Analysis

The General Linear Model, GLM, was used to assess regression and variance using multivariate analysis of variance (MANOVA). When performing overall analyses, transport time was considered as class and independent variable, while all other parameters such as blood parameters (cortisol, glucose, lactate, and creatine kinase), THI, and behaviour parameters (lying, loss of balance, restless and change of positions, vocalization, rooting and fighting) were dependent variables. The model used was:

Table 9 Transport time and distribution of THI in relation to the THI threshold ranges.

Transport time (h)	Transport time interval											
	0-4 (h)				4-8 (h)				8-12 (h)			
	≤ 74	75-78	79-83	≥ 84	≤ 74	75-78	79-83	≥ 84	≤ 74	75-78	79-83	≥ 84
					Winter							
4	100%	-	- - - - - - - - - -									
8	100%	-	-	-	100%	-	-	-	-	-	-	-
12	100%	-	-	-	100%	-	-	-	100%	-	-	-
					Summer							
4	100%	-	- - - - - - - - - -									
8	100%	-	-	-	100%	-	-	-	-	-	-	-
12	75.2%	23.3%	1.5%	-	100%	-	-	-	100	-	-	-

Table 10 Correlation matrix of variables considered in pigs.

	TR	C	CK	L	G	Ls	Rc	Ft	Ly	Vc	Pt	Rt	St
TR	1.00	-0.87	0.94	0.61	0.21	0.95	0.91	0.92	0.99	0.97	0.97	0.89	0.96
C	-0.87	1.00	-0.70	-0.58	-0.42	-0.76	-0.95	-0.95	-0.88	-0.81	-0.90	-0.61	-0.75
CK	0.94	-0.71	1.00	0.40	-0.10	0.93	0.75	0.74	0.91	0.92	0.85	0.94	0.97
L	0.61	-0.58	0.4	1.00	0.62	0.64	0.55	0.68	0.63	0.61	0.59	0.50	0.57
G	0.21	-0.42	-0.1	0.62	1.00	0.17	0.47	0.53	0.28	0.21	0.36	-0.11	0.02
Ls	0.95	-0.76	0.93	0.64	0.17	1.00	0.79	0.84	0.93	0.97	0.90	0.92	0.95
Rc	0.91	-0.95	0.75	0.55	0.47	0.79	1.00	0.97	0.93	0.85	0.96	0.63	0.78
Ft	0.92	-0.95	0.74	0.68	0.53	0.84	0.97	1.00	0.94	0.90	0.96	0.68	0.79
Ly	0.99	-0.88	0.91	0.63	0.28	0.93	0.93	0.94	1.00	0.96	0.98	0.85	0.94
Vc	0.97	-0.81	0.92	0.61	0.21	0.97	0.85	0.90	0.96	1.00	0.93	0.91	0.93
Pt	0.97	-0.90	0.85	0.59	0.36	0.90	0.96	0.96	0.98	0.93	1.00	0.78	0.89
Rt	0.89	-0.61	0.95	0.50	-0.11	0.92	0.63	0.68	0.85	0.91	0.78	1.00	0.94
St	0.96	-0.75	0.97	0.57	0.02	0.95	0.78	0.79	0.94	0.93	0.89	0.95	1.00

$$TR = C\ CK\ L\ G\ Ly\ Ls\ Rc\ Ft\ Vc\ Rt\ St\ Pt + E \quad (4)$$

where the symbols in the equation denote: TR, transport time, E is n × p, and *p* is a dependent variable. Each of the p models can be estimated and tested separately. The other symbols represent cortisol (C), creatine kinase (K), lactate (L), glucose (G), lying (Ly), loss of balance (Ls), restlessness and change of position (Rc), fighting (Ft), vocalization (Vc), sitting (St) and panting (Pt).

The results showed that the model itself was significant ($P < 0.001$). THI was not significant, but all other parameters had a high correlation with transport time, and dependent parameters were partially correlated with each other. A summary of correlation matrix is given in Table 10, where cortisol concentration was negatively correlated with transport time.

Lying, sitting, loss of balance, restlessness and change of position, panting, vocalization, and rooting strongly correlated (0.89-0.99) with transport time. Creatine kinase and lactate correlated with behavioural parameters at levels between 0.75-0.97 and 0.50-0.68 respectively. Fighting behaviour could correlate at different levels with creatine kinase (0.74), lactate (0.68) and glucose (0.53), besides glucose with restlessness and change of position (0.47).

3.7 Cluster Analysis

In the dendrogram shown in Fig. 12, variables Tr-Ly, Ls-Vc, Ck-St, Rc-Ft and L-G formed pair wise clusters with the exception of L-G, they formed the clusters at nearly same level of similarities, 98%-100%. Other single variables like Pt, Rt, and C sequentially added to the clusters. Two other variables namely Pt and Rt could also be added to the existing clusters at around

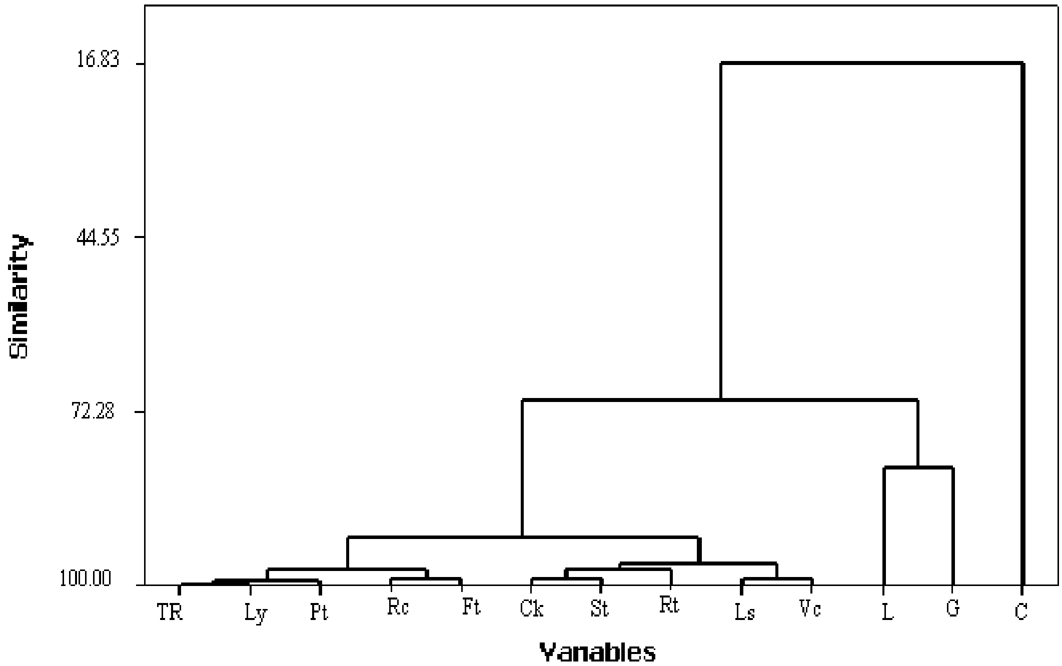

Fig. 12 Dendogram of transport time and stress related behaviour and blood parameters.

95%-98% level of similarities, thus all the variables indicating strong level of similarity where they joined the cluster.

Sub-clusters mainly composed of behaviours (with the exception of CK) formed main cluster at 91.33% level of similarity. Two major clusters which consisted of behaviour and blood parameters (Lactate and Glucose) merged at 72.28% similarity. When C is sequentially added to the main clusters, it occurred at a wider level (16.83%), showing weak level of similarity.

In the clusters, behaviours like lying (Ly), panting (Pt), restlessness and change of position (Rc) and fighting (Ft) are located nearest to transport time (TR) the most similar variables, where as farthest neighbour are the less similar.

4. Discussion

During winter measurements, the values of pH_0 and ultimate pH_{24} ranged from 6.5 to 5.5, which were within the normal range, while summer values varied between 6.29 and 5.99 and exceeded the normal range (Fig. 1). In this study, the ranges of pH_{24} values were higher during summer (5.65 ± 0.17-5.99 ± 0.29) than winter (5.48 ± 0.12-5.58 ± 0.14) (Table 3). The values are within the range 5.8-6.2, where meat is considered to be of lower quality but is not yet classified as DFD meat [34]. During 12 h summer transport, the THI value of 79.24 was noted and this was above the threshold (74). The elevation of pH_{24} might be attributable to the climatic conditions in the vehicle at that time. Seasonal effect in the form of thermal effect has strong influence on the ultimate pH value.

Meat quality of pigs was affected by ambient temperature; higher temperatures led to higher muscle ultimate pH [35]. There is no general agreement on the threshold of DFD. Meat with a pH_{24} value of more than 5.8 or 6 is classified as DFD meat or in some studies 6.2 [36]. In Sweden, 6.2 is applied, and meat with a pH_{24} value between 5.8 and 6.2 is classified as 'meat with low quality'.

Cortisol concentration level was highest at 4 h transport time and generally inversely proportional to transport time (Fig. 4). Cortisol concentration was not positively correlated with stress in swine during transport [37]. When a 15 min was compared with 3 h transport duration, pigs subjected to a shorter journey showed a more intense stress response, thus increased cortisol and lactate levels [38]. Therefore pigs were unable to better adapt to transport conditions for short journey.

Glucose concentrations were highest at 8 (15.3 and 10.3 mmol L^{-1}) and lowest at 12 (11.5 and 3.8 mmol L^{-1}) h transport time for winter and summer experiments, respectively (Fig. 5). Normal values for blood glucose in pigs are between about 4 and 6 mmol L^{-1} but, they can be elevated by stress [39]. In the experiments the reference values of glucose (3.5-6 mmol L^{-1}) were in agreement with the published paper. Averos [40] also reported that glucose level of transported pigs was higher in winter

Concentration level of lactate (4.7-6.2 mmol L^{-1}) increased with an increase in transport time during winter (Fig. 6) and the concentration levels were within the range of reference (4.2-7.3 mmol L^{-1}) values obtained in the study. Lactate levels are very sensitive to stress, the concentrations increased from 2.1 mmol L^{-1} in the pigs showing no evidence of fighting to 9.5 mmol L^{-1} in those showing much fighting [39].

Concentration level of creatine kinase elevated exponentially (R^2 = 0.99) as transport time increased from 4 to 8 and to 12 h. 154 μmol L^{-1} was the maximum value noted at 12 h transport time during summer experiments and the control values varied between 115-129 μmol L^{-1}. The serological profile values of creatine kinase were 105 ± 0.38 and 143.35 ± 0.56 μmol L^{-1} before and after transport, respectively [41].

Behavioural alterations, particularly lying, sitting, rooting, vocalisation, smelling restlessness and change of position and panting were the main effects observed and correlated with transport time (Table 4). Behavioural changes like lying, sitting, restlessness and change of position were in agreement with others research results. Most of the pigs were either sitting or lying, during the whole trip, on long distance transport of up to 24 h [35]. Furthermore, when loading density is higher than 235 kg m^{-2}, not all pigs are able to lie down, hence there is a continual change of position and pigs cannot rest [42]. Vocalization in cattle and pigs are highly correlated with physiological stress [43-45].

Fighting was also among the main observed behaviours and correlated with journey time. Competition for space and mixing from various pens but of the same farm were among the main reasons for fighting activity. Pigs that were mixed with unfamiliar pigs when assembled for sending to slaughter and this usually leads to fighting, particularly between dominant individuals. The consequences are elevations in circulating cortisol, CK and lactate and depletion of muscle glycogen [46].

Values for rooting and vocalization were higher during loading and reversal occurred only during loading. Loading was more stressful than unloading in a scoring system to evaluate stress in cattle [18]. During 12 h transport time behaviours like lying increased as the interval increased. On the other hand, smelling, rooting and vocalization were highest at the first intervals and panting occurred only at 4-8 and 8-12 h intervals. Combination of both high temperature and relative humidity (Fig. 10) or high relative humidity and low temperature (Fig. 9) caused high relative humidity (mean: ≥ 93.8%) for about 7 h in the trip of 8 and 12 h transport time.

When initial relative humidity levels was below 60% (Fig. 11), during loading, there was comparatively slow relative humidity growth and thereafter it decreased rather faster, as the vehicle started moving (compared to higher initial humidity). The possible cause of initial humidity above 60% could be the availability of wet floors and side walls (high moisture) during washing and insufficiently ventilation after washing the vehicle. Length of loading time and number of pigs jointly, had strong influence on the development of climatic conditions in the loading compartments, because the vehicle had a natural ventilation system.

Heat builds up rapidly in stationary vehicle. Vehicles should be kept moving and rest stops should be kept to a minimum [47]. Pigs are sensitive to high temperatures because they are poorly adapted to lose heat unless allowed to wallow, behaviour not possible during transport [44]. Hot weather and humidity are deadly to pigs because they do not have functioning sweat glands. Therefore, special precautionary measures must be taken in hot weather conditions [48].

The overall THI mean values of 4, 8 and 12 h transport time for two seasons remained below the threshold (≤ 74) which is considered normal. But when THI values were subdivided into 3 equal intervals (0-4; 4-8 and 8-12 h) for all transport time, certain summer values surpassed (alert: 23.3% and danger: 1.5%) the normal limit. It occurred during loading event which fell in the interval of 0-4 h (Table 9).

In the dendrogram, which is the output of independent variable transport time (TR) and dependent behavioural and physiological variables, the sub-clusters mostly consisted of behaviours formed main cluster at 91.33% level of similarity. On the other side transport time and blood parameters (L and G) clustered at 86% level of similarity and the two main clusters merged at 72.28%. Therefore degree of association between transport time and behavioural changes was stronger than the blood parameter.

5. Concluding Remarks

The overall conclusion from the study, based on climatic conditions, animal behaviour, stress hormones, and final pH values in carcases, is that an increase in transport time from 4 to 8 h had a higher effect than an increase from 8 to 12 h on the welfare and subsequent meat quality (as determined by pH_{24} values) parameters considered in this study. Most of the values of stress hormones and behaviour increased slightly or remained steady state between 8 and 12 h.

The continuous increase in creatine kinase with an increase in transport time noted in this study indicates increasing muscular fatigue, which could be attributed to restlessness and loss of balance behaviours of animals during transport.

The specific conclusions are:

Cortisol concentration was significantly ($P < 0.001$) elevated during short transport. However the concentration level of cortisol was inversely proportional to transport time;

Glucose concentration increased from 4 to 8 h and thereafter decreased slightly ($P < 0.01$). Lactate concentrations (winter) positively correlated ($P < 0.002$; $R^2 = 1$) with transport time;

Creatine kinase concentration was positively correlated with transport time ($P < 0.01$; $R^2 = 0.99$);

Lying, sitting, rooting, vocalization and smelling were behaviours that scored high values and were positively correlated with transport time ($P < 0.009$). Behaviours like rooting, reversal and vocalization showed that the severity of stress was higher during loading than unloading.

The measured pH_{24} values in carcases showed positively correlated with transport time during summer. The pH_{24} value during summer season for 12 h transport time exceeded 5.8, and the elevation of pH_{24} might be attributable to climatic conditions in the vehicle, where the peak thermo-humidity index (THI) value was also noted to be 79.24 during the 12 h transport time, i.e. above the threshold value of 74. During summer loading caused thermal stress. Inadequate ventilation after washing was the main cause for high initial relative humidity.

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