



Effect of distance and daily periods on heat-stressed pigs and pre-slaughter losses in a semiarid region

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Abstract

Effective planning animal transport is essential to safeguard animal welfare and reduce production losses. Environmental conditions, specifically extreme temperatures in combination with ranges of relative humidity are highlighted as one of the main risk factors for production losses during transport (e.g., fatalities). The majority of research evaluating both welfare and production impacts of pig transport have been primarily undertaken in Europe and North America, which cover a relatively limited range of distinct climates (e.g., temperate, sub-arctic, etc.). As a result, research on pig transport in semi-arid conditions is lacking. In this study, we evaluated the effects of both distance (short, 30 km; and long, 170 km) and transport daily periods (morning, (05:00–11:00); afternoon (12:00–17:00); and night, (23:00–04:00)) on the preslaughter losses and heat stress of pigs in commercial transport in a semiarid region. Across 19 journeys of standard slaughter-weight pig loads (124.0 ± 2.8 kg), 684 focal animals (36 per journey) were evaluated. For each journey, the load's thermal profile (THI_{adj} and enthalpy) and physiological responses of individual pigs were recorded. On arrival at designated slaughterhouses, the percentage pig of non-ambulatory non-injured (NANI), non-ambulatory injured (NAI), death on arrival (DOA), and total losses were recorded. Short journeys in the afternoon were shown to be more detrimental to the thermal comfort of pigs, with higher rectal temperatures recorded. The highest percentage of total losses and DOA occurred in afternoon journeys, irrespective of distance, followed by the morning, with the lowest losses observed in pigs transported at night. Additionally, total losses and DOA were further exacerbated by journey distance, with higher rates observed in short journeys. Higher percentage averages of NANI and NAI were observed in shorter journeys, but daily periods effects were only observed for NANI. These results further demonstrate the welfare and production loss risks associated with journey distance and time of day (representing varying environmental conditions) during road transport of pigs, whilst providing novel data in semiarid conditions. Careful and effective planning for pig transportation is essential to minimize heat stress and production losses. Consideration of the thermal environment on the day of travel, as well as providing flexibility to adjust travel times (e.g., early morning or evening), should help to mitigate risks of heat stress and production losses during pig transport.

Keywords Animal transport · Biometeorology · Thermoregulation · Pre-slaughter

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Introduction

Animal transport is currently under increased scrutiny across all livestock species (Mitchell et al. 2019), including pigs (Faucitano 2018). During road transport, there are several risk factors that can result in physiological stress and/or psychological stress (e.g., distress and pain) in livestock. Examples include feed and water deprivation (Dalla-Costa et al. 2019), inadequate microclimatic conditions (Miranda-de la Lama et al. 2021), mechanical vibrations (Donofre et al. 2014), and human-animal interactions (i.e. handling) (Mota-Rojas et al. 2020).

Pigs are transported by road across the globe and in the majority of continents. This results in journeys occurring across a wide range of environmental conditions (i.e., climate regions) and with further variation in terms of journey profiles (e.g., distances, vehicle design, daily periods, country-specific legislation etc). Typically, road transport of pigs is conducted in non-climate controlled vehicles, which can result, in microclimatic conditions developing inside the vehicle, which are often outside the thermal comfort zone for pigs. Heat stress is considered one of the main factors associated with welfare concerns during transport (e.g. in death on arrivals (DOA), non-ambulatory non-injured animals (NANI)), and production losses (e.g. presence of metabolites that depreciate meat quality (Faucitano 2018), reduced shelf-life by incidence of pale, soft, and exudative (PSE) or dark, hard, and dry (DFD) meat (Dalla-Costa et al. 2019; Romero et al. 2022).

Pigs have limited thermoregulatory ability, with minimal functional sweat glands and a thick layer of subcutaneous adipose tissue which in combination reduces the efficiency of heat loss (Collier and Gebremedhin 2015). Despite pigs being transported by road in most climate regions across the globe, the majority of research has been focused within Europe and North America, with over 80% of the scientific literature published on welfare impacts of road transport in pigs being conducted within these two continents since the 1970s (Mitchell, et al., 2019). These areas do represent a diverse range of climates (e.g., temperate, Mediterranean), but not all. For example, research evaluating welfare and production concerns during pig transport in semi-arid and desert climates is distinctly lacking. These climates are likely to be associated with a high risk of heat stress during road transport and associated preslaughter losses in pig transport. Therefore there is a potential greater risk to the welfare of pigs and the profitability of carriers in a semiarid region, due the thermal conditions most noxious to animal homeostasis, and use of vehicles that are not environmentally controlled compared to pigs transported in temperate climates (Dos Reis et al. 2015; Dalla-Costa et al. 2019; Machado et al. 2021a, b).

In this study we aim to assess of thermal profiles in a semiarid region, whilst taking into account local vehicle

and journey parameters (e.g., transport distance and journey time), in order to contribute to the development of guidelines and recommendations specific to the road transport of pigs in semi-arid conditions, resulting in the improvement of both the short- and long-term welfare impacts observed in pigs during and following transport, as well as reducing production losses. We hypothesize that the (i) pig transport in the afternoon, where ambient temperatures are likely to be higher in a semiarid region, represents a higher risk to pig welfare and production losses compared to transport occurring at night or in the morning, and (ii) in long journeys would result in greater welfare compromise and production losses compared to shorter journeys, as the pigs will be exposed to microclimatic conditions, out with the pig thermal comfort zone for longer periods.

To test these hypotheses, we assessed the both individual and combined effects of distance (30 km and 170 km) and transportation daily periods (morning, afternoon, and night) on the incidence of pig heat stress and associated production losses, during transportation by road in a semiarid region.

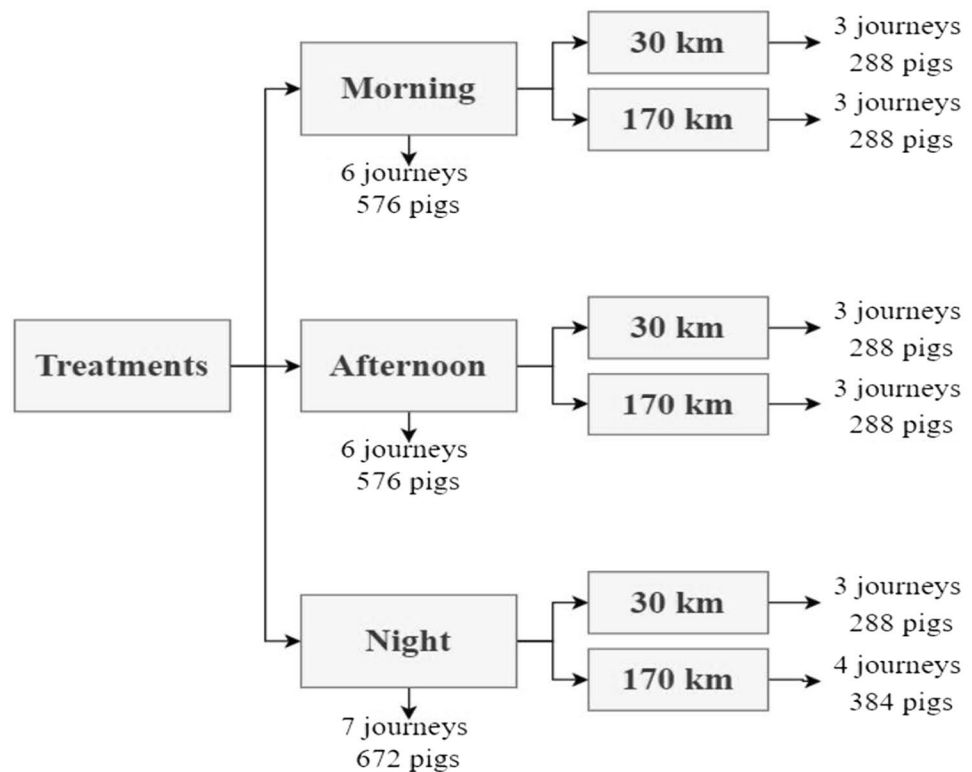
Materials and methods

All procedures were performed following the Ethics Committee's guidelines on the Use of Animals of the Agrarian Science Center of the Federal University of Ceará (Process number 4244290319).

Animals, treatments, and management

This study was performed with a total of 1824 crossbred pigs (Large White × Landrace × Duroc), of both sexes and approximately 165 days of age. From the 1824 transported pigs, a subsample of 684 focal animals, with a bodyweight of 124.0 ± 2.8 kg (min = 119.5 kg and max = 127.2 kg), were selected by random block sampling by sex (only female) and by location in the load (3 pigs per compartment, totaling 36 animals per journey). Focal animals were identified with paint markings on the dorsal and auricular region in the farm stall, 80 ± 15 min before loading, to receive the protocol for collecting physiological parameters.

All animals were sourced from a single farm, located in the city of Maracanaú, Ceará, Brazil ($3^{\circ}54'46.4''$ S $38^{\circ}39'19.2''$ W and 43-m altitude). Thus, two commercial routes were selected, corresponding to distances, short (30 km) and long (170 km), with both terminating, respectively, at commercial slaughterhouses in the cities of Caucaia, Ceará, Brazil ($3^{\circ}44'4''$ S $38^{\circ}39'23''$ W and 24 m altitude), and Morada Nova, Ceará, Brazil ($5^{\circ}06'24''$ S $38^{\circ}22'21''$ W).

Fig. 1 Scheme of the investigated treatments

W and 52-m altitude). In this study, a total of 19 journeys were monitored, with nine short journeys (30 km: from Maracanaú to Caucaia) and ten long journeys (170 km: from Maracanaú to Morada Nova). The schedules of the journeys were divided into three shifts: morning (05:00 to 11:00), afternoon (12:00 to 17:00), and night (23:00 to 04:00), resulting in the experimental scheme presented in Fig. 1.

The journeys monitored occurred during the between February 2nd and 20th 2019 (local summer season). The animal handling pre-loading and transport was

standardized across all journeys and according to the company's commercial standard. Feeding of the animals was suspended 120 ± 20 min (min 94 min and max 142 min) before loading started. At loading, all animals were guided with the help of flags. Pre-molded loading concrete ramps with a 30° slope for loading of the lower deck and a metal ramp (~ 3 m, slope 58°) for loading on the upper deck.

During loading pigs, “load cooling” occurred, which involved loaded pigs in the truck being wetted with water by a farm employee using a hose. This is a standard practice of region, aiming to reduce the impact related to heat stress during transport (Pinheiro et al. 2020). The “load cooling” was performed randomly along the truck body, and the average amount of water used ($\pm$ SE) was 2852 ± 210 l/load (min = 2055 l/load and max = 3468 l/load).

All journeys were performed in a Ford® cargo 1519 truck, with a two-story Triel®—HT body model, containing six compartments (height = 0.95 m, length = 2.40 m, and width = 1.30 m, totaling area of 3.20 m^2) per deck/level and a load capacity of 13 tons (Fig. 2). As standard local practice (Northeast of Brazil in 2019) the vehicle was not equipped with an air conditioning system or environmental controls, and the compartments did not contain any type of bedding or drinking water supply system. National decrees on the transit of animals became enforced as of 2021 (CONTRAN, resolution N° 791 2020), this study was conducted prior to their enforcement. All journeys were

**Fig. 2** Trailer used to transport pig

Table 1 Averages speed, loading, transport and unloading times of the transport operations

Period	Distance (km)	Averages speed (km/h)	Time (min)		
			Loading	Transport	Unloading
Morning	30	68.51 ± 3.9	27.67 ± 2.5	26.33 ± 2.5	20.33 ± 1.5
	170	75.98 ± 2.3	52.67 ± 3.0	134.33 ± 2.0	22.00 ± 4.1
Afternoon	30	65.20 ± 3.6	27.33 ± 2.5	27.67 ± 1.0	29.00 ± 1.5
	170	74.28 ± 1.1	55.67 ± 6.0	137.33 ± 2.0	28.00 ± 2.0
Night	30	77.09 ± 4.6	46.67 ± 3.5	23.33 ± 4.0	35.67 ± 1.5
	170	87.40 ± 2.0	64.25 ± 4.3	116.75 ± 2.1	30.25 ± 2.7

undertaken on paved roads following fixed routes and, were conducted by a same truck driver, and had a load density of 290 kg/m². The average truck speed, loading, transport, and unloading times are presented in Table 1.

Thermal assessment

External air temperature and relative humidity meteorological data was obtained from weather stations 82,397-For-taleza (3°49'12" S 38°32'24" W and 29.89-m altitude) and 82,588-Morada Nova (5°8'24" S 38°21'36" W and 45.02-m altitude) of the National Institute of Meteorology of Brazil for the durations of all journeys. Inside the trailer, air temperature and relative humidity data were recorded every 10 min by data loggers (Onset, U23-001 HOBO Pro v2, Massachusetts, USA) positioned in the center of each of the 12 compartments at pig height.

The characterization of the microclimatic of the trailer was performed by using the specific enthalpy (Rodrigues et al. 2011) by Eq. 1. The cooling effect of the airflow caused by vehicle speed was evaluated using the THI adj (Mader et al. 2006) by Eq. 2. The wind speed and solar radiation of the trailer was obtained using computational fluid dynamics (CFD) techniques. Details of the CFD simulation are provided in the “Supplementary information.” The boundary conditions used in the simulation for each treatment were established with the information collected in the actual monitoring of the journeys.

$$H = 1.006 \times TA + \frac{RH}{Pb} \times 10^{7.5TA(237.3 + TA)^{-1}} \times (71.28 + 0.052 \times TA) \quad (1)$$

$$THI_{adj} = 4.51 + THI - (1.992 \times WSPD) + (0.0068 \times RAD) \quad (2)$$

where H is the specific enthalpy (kJ/kg dry air); THI_{adj} is the THI adjusted to WSPD and RAD; TA is the air temperature (°C); RH is the relative humidity (%); Pb is the local barometric pressure (mmHg); $WSPD$ is the wind speed; RAD is the solar radiation; and THI is the temperature-humidity index: $THI = 0.8 \times TA + \left[\left(\frac{RH}{100} \right) \times TA - 14.4 \right] + 46.4$, by Mader et al. (2006).

Animal parameters

The physiological measures of focal pigs were rectal temperature (°C), skin temperature (°C), respiratory rate (mov/min), blood lactate (mg/dL), and salivary cortisol (ng/mL). The measurements were made on average 45 ± 12 min before loading and 25 ± 15 min after unloading the animals, at the slaughterhouse's waiting pen (i.e., lairage). In the statistical modelling, the mean of these measures per journey was considered in the data set.

The measurements of respiratory rate and skin temperature was performed before restraining the pigs and without physical interference (as required for other physiological measures described below). Skin temperature was obtained by thermal imaging from a thermographic camera (TiS10, Fluke, 4800 pixels resolution), by adjusting the environmental temperature at the time of image recording, and emissivity of biological tissues $\epsilon = 0.98$ (Machado et al. 2021c). Thermal images were obtained with a fixed distance of 0.50 m between the animal and the observer. All thermal images were analyzed in the Smart-View 4.3 software.

Respiratory rate was measured by two trained specialists, observing the animals' flank movement for 30 s (Machado et al. 2020). The two observers recorded the respiratory rate measurements simultaneously; therefore, the a mean obtained by the experts, was calculated for each animal based on the two measures obtained provided by the experts, which was subsequently used in statistical modelling.

Following non-restraint measures (i.e., respiratory rate and skin temperature), pigs were lightly restrained and briefly (max 2 min) in a supine position for measuring rectal temperature, salivary cortisol, and blood lactate concentration. All samples were measured from each animal within a range of 3 ± 1 min. Rectal temperature was measured using a skewer thermometer with accuracy ± 0.5 °C (− 10 to 100 °C, AK05, AKSO), inserted directly into the animal's rectum at a depth that the bulb was in contact with the rectal mucosa (Pereira et al. 2022).

Lactate was determined by the portable analyzer Lactate Scout 4 (EKF Diagnostic GmbH, Cardiff, Wales, UK), according to Rocha et al. (2015). Blood samples from the pigs' marginal ear vein were collected by a veterinarian using a retractable needle (25G × 16 mm). This procedure was followed by hemostasis and cleaning of the animals' ears by the veterinarian to avoid sequelae. A drop of blood was then used on a sample strip (two replicates per animal) and inserted into the portable analyzer, with the results provided after 15 s.

Salivary cortisol was determined using the Elisa Salimetrics kit (NeogenCorp. Lexington, KY, USA), as described by Dalla-Costa et al. (2009). Saliva was collected using a plastic swab with a sterile Stuart medium inserted into an Eppendorf tube. The plastic swab was kept in the animals' mouths for about 20 ± 3 s. The samples were stored in a freezer at -20°C for approximately 50 min until laboratory analysis was performed.

Productivity losses

During loading, an observer recorded the time required loading the animals on to the truck. The total time of the journey (defined as wheels turning) and average speed of transport were also recorded. At the completion of the journey, pigs were unloaded, with an observer recording the time needed to complete unloading and move pigs to the slaughterhouse's waiting pens.

Another observer recorded the number of pigs classified as non-ambulatory non-injured (NANI), animals panting or refusing to stand, but no apparent injury, trauma, or illness; non-ambulatory injured (NAI), injured animals that cease their movements or have their locomotion compromised; and the occurrence of deaths on arrival (DOA) from loads according to Ritter et al. (2006). The total production losses of each load was estimated by summing the percentage of NANI pigs, NAI pigs, and DOAs (Ritter et al. 2009).

Statistical analysis

The research was a factorial design, with distance and daily periods as the main factors. The experimental unit was each journey and each focal pig was considered an observation. The hypothesis of normality of the residuals of the data was verified by the Kolmogorov test ($P < 0.05$). An ANOVA using the PROC GLM of the Statistical Analysis System (SAS® Inst. Inc., Cary, NC), was performed according to the following model (3). Tukey–Kramer adjustments were used to compare treatment means ($P < 0.05$).

$$Y_{ijk} = \mu + D_i + P_j + I_{ij} + S_k + \varepsilon_{ijk} \quad (3)$$

where Y_{ijk} is the dependent variable, μ is the overall mean, D_i is the fixed effect of the distance, P_j is the fixed effect of the daily periods, I_{ij} is the effect of the interaction between the i_{th} distance and the k_{th} daily periods, S_k is the random effect of the collection week, and ε_{ijk} is the residual effect which includes the other sources of variation.

Principal component analysis was performed using the PRINCOMP procedure to characterize the investigated treatments. Initially, the dataset composed of the means of the variables of each treatment were standardized ($\mu = 0$; $s^2 = 1$), aiming to eliminate the influence of the different units of measurement of the variables. The criterion for choosing the first two principal components was based on eigenvalues greater than one. That done, a biplot was prepared by the PRINQUAL procedure to analyze correlations and multivariate trends.

Results

Variations of air temperature, relative humidity, and environmental enthalpy during the study are shown in Fig. 3. During the journeys monitored, ambient temperature reached

Fig. 3 Environmental variation: averages and standard deviation of air temperature, relative humidity, and environmental enthalpy during journeys

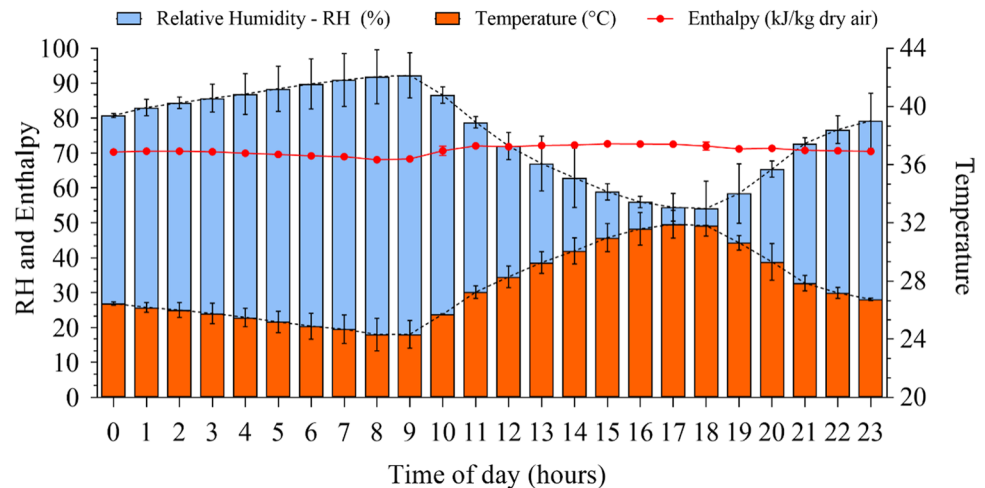


Table 2 Averages ($\pm$ SE) of enthalpy, and temperature-humidity index adjusted (THI_{adj}) of trailer

Parameters	Distance	Transport of period			Average
		Morning	Afternoon	Night	
Enthalpy (kJ/kg dry air)	30 km	79.52 $\pm$ 0.62	84.24 $\pm$ 0.48	78.95 $\pm$ 0.78	80.90
	170 km	83.53 $\pm$ 0.60	82.46 $\pm$ 0.35	75.90 $\pm$ 0.53	80.63
	Average	81.52 ^B	83.35 ^A	77.42 ^C	
THI_{adj}	30 km	74.43 $\pm$ 0.46 ^{Bb}	87.28 $\pm$ 0.88 ^{Aa}	59.84 $\pm$ 1.28 ^{Ba}	73.85 ^a
	170 km	70.25 $\pm$ 0.67 ^{Aa}	78.35 $\pm$ 1.47 ^{Ab}	55.98 $\pm$ 1.60 ^{Bb}	68.19 ^b
	Average	72.34 ^B	82.82 ^A	57.91 ^C	
<i>P</i> value		Period (P)	Distance (D)	P $\times$ D	CV ¹ (%)
Enthalpy		< 0.001	0.183	0.056	3.80
THI_{adj}		< 0.001	< 0.001	0.016	12.37

Averages followed by equal letters (in comparison consider: horizontal uppercase and vertical lowercase) do not differ statistically from each other by the Tukey–Kramer test ($P < 0.05$)

31.90 °C at 17:00, with minimum of 24.32 °C at 08:00, revealing amplitude greater than 7 °C. The relative humidity of the air remained between 54.10 and 92.29% during the study, with a maximum at 09:00 h. The daily enthalpy remained between 72.65 and 68.14 kJ/kg dry air, but reached 72.09 kJ/kg dry air at 11:00 and remained high until 18:00 h (> 72 kJ/kg dry air by Silva-Miranda et al. 2012).

The results showed significant effect of daily periods ($P < 0.001$), for enthalpy inside the trailer. The highest

enthalpy averages ($P < 0.001$) recorded were observed during transport in the afternoon, regardless of distance (Table 2). A significant effect of the interaction of distance and daily periods ($P = 0.016$) was observed for THI_{adj} , with the highest THI_{adj} observed during transportation afternoon, followed by the morning and the night. Furthermore, the results showed that THI_{adj} was consistently higher in short journeys compared to longer journeys, irrespective of daily periods (Table 2).

Table 3 Averages ($\pm$ SE) of physiological parameters of pigs transported

Parameters	Distance	Transport of period			Average
		Morning	Afternoon	Night	
Rectal temperature (°C)	30 km	39.31 $\pm$ 0.66 ^{Ba}	40.19 $\pm$ 0.27 ^{Aa}	38.97 $\pm$ 0.28 ^{Ca}	39.49 ^a
	170 km	39.25 $\pm$ 0.64 ^{Aa}	39.55 $\pm$ 0.23 ^{Ab}	38.60 $\pm$ 0.15 ^{Bb}	39.13 ^b
	Average	39.28 ^B	39.80 ^A	38.78 ^C	
Respiratory rate (breaths/minute)	30 km	84.00 $\pm$ 3.00	92.00 $\pm$ 3.00	68.00 $\pm$ 2.00	81.00
	170 km	80.00 $\pm$ 2.00	94.00 $\pm$ 2.50	72.00 $\pm$ 3.00	82.00
	Average	82.00 ^B	93.00 ^A	70.00 ^C	
Skin temperature (°C)	30 km	39.24 $\pm$ 0.50 ^{Ba}	40.47 $\pm$ 0.90 ^{Aa}	39.09 $\pm$ 0.48 ^{Ba}	39.60 ^a
	170 km	39.31 $\pm$ 0.26 ^{Aa}	39.51 $\pm$ 0.57 ^{Ab}	38.66 $\pm$ 0.36 ^{Ba}	39.16 ^b
	Average	39.27 ^B	40.04 ^A	38.87 ^C	
Salivary cortisol (ng/mL)	30 km	33.63 $\pm$ 0.69 ^{Ba}	36.85 $\pm$ 0.80 ^{Aa}	31.86 $\pm$ 1.12 ^{Ca}	34.11 ^a
	170 km	32.49 $\pm$ 0.85 ^{Bb}	33.80 $\pm$ 0.96 ^{Ab}	30.97 $\pm$ 0.68 ^{Ca}	32.42 ^b
	Average	33.06 ^B	35.32 ^A	31.41 ^C	
Lactate (mg/dL)	30 km	71.33 $\pm$ 0.86	75.25 $\pm$ 1.23	69.19 $\pm$ 1.00	71.92 ^a
	170 km	67.13 $\pm$ 1.26	71.33 $\pm$ 1.55	64.13 $\pm$ 1.30	67.53 ^b
	Average	69.23 ^{AB}	73.29 ^A	66.66 ^B	
<i>P</i> value		Period (P)	Distance (D)	P $\times$ D	CV ¹ (%)
Rectal temperature		< 0.001	< 0.001	0.034	3.84
Respiratory rate		< 0.001	0.823	0.081	14.20
Skin temperature		< 0.001	< 0.001	< 0.001	4.34
Salivary cortisol		< 0.001	< 0.001	< 0.001	6.80
Lactate		< 0.001	< 0.001	0.379	8.56

Averages followed by equal letters (horizontal uppercase and vertical lowercase) do not differ statistically from each other by the Tukey–Kramer test ($P < 0.05$)

Table 4 Averages ($\pm$ SE) of the percentage of non-ambulatory non-injured (NANI), non-ambulatory injured (NAI), deaths on arrival (DOA), and total loss during transport operations

Parameters	Distance	Transport of period			Average
		Morning	Afternoon	Night	
NANI (%)	30 km	0.69 $\pm$ 0.03	2.08 $\pm$ 0.04	0.69 $\pm$ 0.03	1.15 ^a
	170 km	1.04 $\pm$ 0.02	1.04 $\pm$ 0.02	0.34 $\pm$ 0.01	0.80 ^b
	Average	0.86 ^B	1.56 ^A	0.50 ^B	
NAI (%)	30 km	0.69 $\pm$ 0.03	1.38 $\pm$ 0.04	0.69 $\pm$ 0.03	0.92 ^a
	170 km	0.34 $\pm$ 0.01	0.69 $\pm$ 0.03	0.69 $\pm$ 0.03	0.55 ^b
	Average	0.51	1.0	0.66	
DOA (%)	30 km	0.69 $\pm$ 0.03 ^{Ba}	1.04 $\pm$ 0.03 ^{Aa}	0.34 $\pm$ 0.01 ^{Ca}	0.69
	170 km	0.34 $\pm$ 0.01 ^{Ab}	0.34 $\pm$ 0.01 ^{Ab}	0.00 $\pm$ 0.00 ^{Bb}	0.24
	Average	0.51	0.68	0.17	
Total loss (%)	30 km	2.07 $\pm$ 0.03 ^{Ba}	4.50 $\pm$ 0.04 ^{Aa}	1.72 $\pm$ 0.02 ^{Ca}	2.76
	170 km	1.72 $\pm$ 0.02 ^{Bb}	2.07 $\pm$ 0.02 ^{Ab}	0.97 $\pm$ 0.02 ^{Cb}	1.60
	Average	1.89	3.28	1.34	
<i>P</i> value		Period (P)	Distance (D)	P $\times$ D	CV ¹ (%)
NANI		< 0.001	< 0.001	0.160	3.84
NAI		0.162	0.024	0.073	9.20
DOA		< 0.001	0.048	< 0.001	4.34
Total loss		< 0.001	< 0.001	< 0.001	8.56

Averages followed by equal letters (in comparison consider: horizontal uppercase and vertical lowercase) do not differ statistically from each other by the Tukey–Kramer test ($P < 0.05$)

Table 5 Coefficients and total variation explained by each principal component

Variables	Principal Component (PC)	
	PC1	PC2
Enthalpy	0.257	0.376
Temperature-humidity index adjusted—THI _{adj}	0.309	0.192
Transport time	−0.120	0.687
Rectal temperature	0.323	0.100
Respiratory rate	0.257	0.369
Skin temperature	0.321	0.031
Salivary cortisol	0.324	−0.099
Lactate	0.307	−0.177
NANI—pigs non-ambulatory non-injured	0.308	0.067
NAI—pigs non-ambulatory injured	0.247	−0.350
DOA—deaths on arrival	0.302	−0.208
Total loss	0.316	−0.125
Eigenvalue	9.3996	1.6392
Variation (%)	78.33	13.66
Accumulated variation (%)	78.33	91.99

In this study, a significant interaction of the distance $\times$ daily periods was found for the rectal temperature ($P = 0.034$), skin temperature, and salivary cortisol concentrations of the pigs (both $P < 0.001$), as shown in Table 3. Pigs transported in the afternoon had a higher rectal temperature, skin temperature, and salivary cortisol

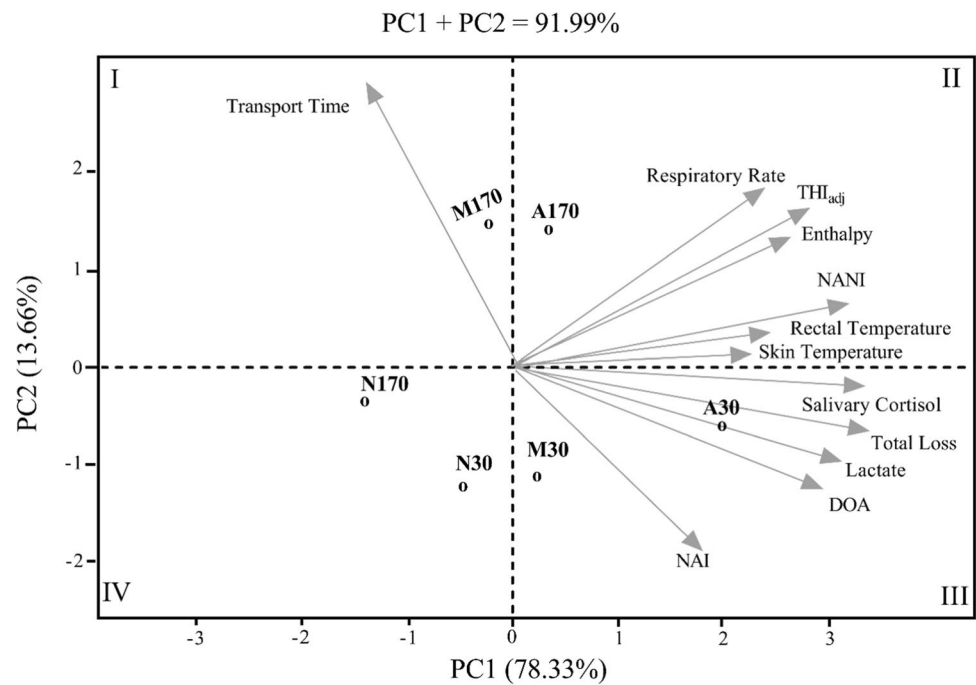
concentrations compared morning or at night, and within daily periods, all measures were, significantly higher in shorter journeys compared to longer journeys. The average respiratory frequency of pigs was higher ($P < 0.001$) in the afternoon journeys (93 breaths/min), followed by morning (82 breaths/min), and night (70 breaths/min), but no differences based on journey distance.

Pigs transported in the afternoon also showed increased blood lactate concentration (+6.63 mg/dL) compared to pigs transported at night ($P < 0.001$), but no difference was detected to pigs transported in the morning. Significant differences also were found for journeys, with pigs transported for 30 km (short) had the greatest blood lactate concentrations (+4.39 mg/dL) compared to pigs transported for 170 km (long) (Table 3).

A significant interaction between distance and daily periods was found for percentage DOA and total loss (both, $P < 0.001$). The highest averages occurred in journeys in the afternoon, followed by the morning, compared to the night period, significantly higher in short journeys. Percentage of pigs classified as NANI were higher in shorter journeys ($P < 0.001$), especially during transport in the afternoon ($P < 0.001$), with increase of 0.69% and 1.06% in relation to the morning and night periods, respectively. For the percentage of pigs classified as NAI, higher averages were observed in the shorter journeys ($P = 0.024$), with no significant effect ($P = 0.162$), for the period of the journeys (Table 4).

Principal component analysis (PCA) allowed for the characterization of the investigated treatments. PCA

Fig. 4 Biplot of the principal component analysis: characteristics of the investigated treatments. THI_{adj} = temperature-humidity index adjusted; NANI = non-ambulatory non-injured; NAI = non-ambulatory injured; DOA = deaths on arrival



explained 91.99% of the total structure of variance and covariance (PCA 1 (78.33%), PCA 2 (13.66%)), between physiological parameters of heat stress and production losses as a function of distances and daily periods of transport (Table 5). The transport in the afternoon period associated with short journeys was moved to quadrant III by the principal component axis in the positive direction, being superimposed by the mean vectors of total loss, NAI, and DOA concentration of salivary lactate and cortisol (Fig. 4).

Discussion

Worldwide, there is growing public interest and concern around animal welfare standards for livestock animals, which has increased pressure on the industry to improve its production techniques. Examples in pig production include the banning of gestation stalls in Europe and a drive for adoption of free-farrowing systems (Yunes et al. 2018), as well as enriching the pig breeding environment and improving microclimate and cooling systems in pig houses (Godyn et al. 2020).

One animal welfare issue that is receiving considerable attention in investigations in support of the pork industry is transport. The present study is the first to describe the effects of distance and transportation daily periods on the incidence of pig heat stress and associated production losses, during transportation in a semiarid region. Our results indicate that the transport in the afternoon was more harmful to the thermal comfort of pigs and

was observed to have the highest enthalpy values from the trailer's microclimate profile. Critically, this primary finding illustrates the importance of considering environmental conditions (e.g. environmental enthalpy), when choosing when to transport pigs in a semi-arid region, as this mitigation strategy through careful journey planning could prevent pigs being transported during the hottest parts of the day (i.e. the afternoon) and minimise the risk of heat stress and associated production losses by opting to conduct journeys during the cooler periods (e.g., early morning or evening).

The observed increases in the majority physiological measures in pigs transported in the afternoon confirm the animals were suffering from heat stress, albeit with severity of heat stress dependent on compartment location (Machado et al. 2021a). Pigs that are suffering from heat stress have a greater demand on the physiological mechanisms of thermoregulation, in an attempt to maintain homeostasis (Mota-Rojas et al. 2021). Previous studies have observed that trailer compartments resulting in most harmful microclimate conditions (i.e., higher THI or enthalpy), correspond to the places with the highest temperature of the gastrointestinal tract in pigs (Pereira et al. 2018), rectal temperatures, and higher respiratory rate (Machado et al. 2021a), confirming a direct relationship between the quality of the trailer microclimate and the onset of heat stress in pigs during a journey.

In this study, significant differences also were documented for distance of transport. Transport during the hottest times of the day were also associated with elevated salivary cortisol concentration, suggesting a greater activity of the

adrenocortical system. Besides, the higher lactate levels in these animals indicate increased anaerobic metabolism, suggesting increased fatigue and muscle exhaustion (Machado et al. 2021d). Surprisingly, our data show that the transport in the afternoon period associated with short journeys was most critical scenario for the animal welfare of the pigs and the productive losses (Fig. 3). The relationship between negative welfare states (including associated production losses) and journey distance does not appear to be linear, but a complex combination of multiple factors defining the journey profile, animal profiles and environmental conditions.

This result allows us to suggest the hypothesis that this occurred because the longer journeys may have allowed the pigs to recover from the stress of loading, and to adapt to transport conditions, due to the longer operating time (Table 1). Among these conditions are the heat stress, new smells and noises, mixing with unknown animals, deprivation of food, and handling of loading/unloading, among other factors (López-Arjona et al. 2020). All times during the pre-slaughter handling, pigs may experience stress. However, a number of studies have already shown the great risk during pigs loading and unloading (Goumon and Faucitano 2017; Faucitano 2018; Grandin 2021; Romero et al. 2022). Thus, longer journeys may have acted as a “rest period” for the stress of pigs loading and unloading, similar in effect to a waiting time at the slaughterhouse.

Overall, as all pre- and post-transport practices (e.g., handling, sampling methods, infrastructure, employees, truck, among other factors.) were identical in our study; these observed differences between transport distances seem genuine and correspond to previous works (Haley et al. 2008a and Aradom et al. 2012). Therefore, our results show that, in fact, depending on the level of stress during pre-slaughter management, pigs may not be able to recover during short journeys, especially if associated with high temperatures and non-environmentally controlled vehicles, negatively affecting the animal welfare, and increasing production losses (Dalla-Costa et al. 2019).

There is limited data available on daily periods effects during pig transportation, however journey distance effects observed corresponded to previous works. Haley et al. (2008a), who reported that every 50 km increase in distance can decrease DOA about 0.81 times, in addition to the reduced risk of DOA rates on journeys with distances over 134 km. As reviewed by Sutherland et al. (2014), the transport duration has the potential to influence the well-being of pigs in transit. In slaughter weight pigs, higher mortality rates are recorded during short (30 min to 90 min) transport times than transport times greater than 3 h. However, after ~ 18 h of transit, dehydration and loss of body weight (Machado et al. 2016), can drive pigs into a catabolic state to maintain homeostasis (Sutherland et al. 2014).

Similarly, Aradom et al. (2012) checked that pigs' cortisol concentration level was highest at 4-h transport time (160 nmol L⁻¹) comparing to times of 9 h (140 nmol L⁻¹), 21 h (120 nmol L⁻¹), and 23 h (~ 110 nmol L⁻¹), showing an inversely proportional relationship to transport time. Perez et al. (2002) concluded that under normal commercial conditions in Spain, pigs subjected to shorter journeys (15 min) showed a more intense stress response compared to pigs subjected to longer journeys (3 h). These authors verified 19.02 mg/mL and 29.46 ng/mL increase in blood lactate and cortisol, respectively, and losses in meat quality (0.09 and 0.17 reduction in pH of the *Longissimus thoracis* and *Semimembranosus* and discoloration of meat) when the animals were immediately submitted to slaughter upon arrival at the slaughterhouse.

In pig transportation, Haley et al. (2008b) and Correa et al. (2013) reported higher losses during the Canadian summer, with the highest rates of DOA being recorded during August (about 0.40%) when the maximum recorded outdoor air temperature was 33.6 °C (Haley et al. 2008b). Dalla-Costa et al. (2019) evaluating the risk factors associated with pre-slaughter losses in swine in southern Brazil showed that the average temperature increased the probability of total losses in transport, especially > 20 °C. The same impact caused by heat stress on DOAs was reported during winter and Brazilian summer, during the afternoon transport shift of live broiler (Santos et al. 2020).

Concerning the pig cooling during transport, we know that the displacement of the truck generates a vacuum (negative gauge pressure) in the compartments close to the truck cabin, resulting in less airflow in the front region of the trailer (Gilkeson et al. 2009; Machado et al. 2021a), and consequently a reduced rate of heat removal from this location (Pinheiro et al. 2022), forming “thermal cores,” where enthalpy values are the highest (Machado et al. 2021b). The results of the THI_{adj} in this study suggest that the convective heat exchange rate of pigs, caused by vehicle speed, is greater during night transport, especially on 70-km journeys (Table 2).

The reason for this is the exposure to solar radiation during transport in the morning and afternoon shifts, and the higher average speed of the truck on during night transportations (Table 1). In this situation, the lowest environmental enthalpy and the better traffic condition during night transportation generates an adequate thermal gradient for pig's convective heat exchange. However, higher speeds can affect the balance of the pigs, increasing risk of pigs classified as NAI (Dalla-Costa et al. 2019), and being at greater risk of falling or being bumped about during transport. Both of which result in poor pig welfare from potential injuries, but also the fear

and anxiety associated with the unpredictable and unstable environments and potential motion sickness. Consequently this may result in increases of production losses through DOAs, as well carcass and meat quality down-grades (Faucitano 2018).

It is important to note that in this study, the highest percentage of pigs classified as NAI, was observed in the shortest journeys, which were associated with shorter loading times (Table 1). Despite efforts to standardize handling methods across all journeys, the shorter loading times observed in the shorter journeys for the same number of animals may have resulted in a confound of handling. Rough handling can cause higher accident rates during these operations (Dalla-Costa et al. 2019; Goursoot et al. 2019). Shorter loading times for pigs on shorter trips can be attributed to the farm's attempt to optimize the number of journeys made in the day to reduce the daily rates allocated to a single batch and minimise costs.

In this study, local practices for stocking density and “load coiling” were followed. However, the high density of transport (290 kg/m²) and the practice of “wetting” the load and pigs during loading may have contributed to additional the DOAs and production losses. The European Union recommends a stocking density during transport of up to 235 kg/m², because above that, there is inadequate space, preventing all pigs to lie down and rest simultaneously, as well as being continually physically disturbed by other pigs due to cramped conditions (Rioja-Lang et al. 2019). Studies have shown higher stocking densities during transport can result in higher DOAs and other production losses as well as meat quality impacts (Ritter et al. 2009).

Utilising a cooling system through water misting with forced ventilation have been shown to be effective in improving the internal thermal environment of the trailer and the thermal comfort of pigs (Fox et al. 2014; Pereira et al. 2018). However, the practice of “wetting” the load during loading, which is common in tropical and semiarid regions, may be counterproductive and could result in heat stress and negative pig welfare states, especially if the vehicle remains stationary after “wetting” (Pereira et al., 2018; Rioja-Lang et al., 2019). Excessive wetting with a hose, rather than “misting” may have contributed to the accumulation of excess water in the truck body, increasing the humidity in the vehicle and generating heat stress inducing microclimates. The excess of water may have also limited the heat exchange of pig, which is based on the evaporative mechanism and behavioral heat responses, such as increased panting. Adequate personnel training on pre-slaughter handling, especially in loading and unloading animals, is essential to reduce the risk of injuries and heat stress to protect animal welfare and avoid losses (Dalla-Costa et al. 2019; Benincasa et al. 2020).

Conclusions

This study contributes to a knowledge gap on the animal welfare risks during the transport of pigs by road in semi-arid regions. The results demonstrate animal welfare was most compromised when transport was conducted during the warmest periods of the day (e.g. afternoon), resulting in higher production losses. Additionally, production losses were also associated with short distance journeys (30km) compared to longer journeys (170km), suggesting that longer distances enable the pigs to adapt to the social and physical environment they are exposed to. Whilst, also allowing for adequate recovery from stress caused by handling at the loading stage. This study's findings suggest that pigs should not be transported during the hottest periods of the day (e.g. afternoon) in semi-arid regions and effective journey planning should be utilised to identify the coolest times dependent on vehicle and animal parameters as well as environmental conditions in order to transport pigs with minimal risks of heat stress and associated production losses.

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Declarations

Conflict of interest The authors declare no competing interests.

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