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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research
Article Title (within 20 words without abbreviations)	Effects of driving style and bedding in pigs transported to slaughterhouse in different temperatures
Running Title (within 10 words)	Effects of driving style and bedding on animal welfare
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This work was carried out with the support of “Cooperative Research program for Agriculture Science & Technology Development (Project No. PJ016214)” Rural Development Administration, Korea.

Acknowledgements	Not applicable.
Availability of data and material	All data generated or analyzed during this study are included in this published article.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Cho JH, Song DC. Data curation: Song DC, Oh HJ. Formal analysis: Chang SY, Park SH, Jeon KH. Methodology: Kim KH, An JW. Software: Chang SY. Validation: Lee JH, Oh HJ. Investigation: Cho JH, Song MH, Kim HB. Writing - original draft: Cho JH, Kim HB, Song DC, Lee JH, Kim KH, Song MH. Writing - review & editing: Cho JH, Kim HB, Song DC, Lee JH, Kim KH, Song MH, Oh HJ, Chang SY, An JW, Park SH, Jeon KH.
Ethics approval and consent to participate	The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-1740-22-02).

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Abstract

Animal welfare during transport became an largely issue because of increasing demand for improved animal welfare standards. Most studies on the animal welfare during transportation have concentrated on the atmosphere and the temperature of the truck compartments. Thus, the objective of study was to collect and quantify three axis acceleration and determine the effect of bedding for transporting pigs from farm to slaughterhouse. A total of 2,840 crossbred fattening pigs with a live weight of approximately 115 kg were used. They were raised in the same commercial farms and transported to the same commercial slaughterhouse. A 3×2×2 completely randomized factorial design was used to investigate effects of rubber type bedding (bedding or non-bedding) and two levels of driving style (aggressive or normal) in three different time periods with different outside temperatures. Air temperature treatments were as follow: HT, high temperature (higher than 24°C); LT, low temperature (lower than 10°C); NT, normal temperature (10°C to 24°C). In our experiment, pigs transported under aggressive driving style showed lower ($p < 0.05$) pH and WHC than those transported under normal driving style. Pigs transported under normal driving style showed a lower percentage of DL ($p < 0.05$) than those transported with an aggressive driving style. Also, transported with bedding showed higher ($p < 0.05$) lying behavior but lower ($p < 0.05$) sitting behavior than those transported without bedding. Pigs transported under normal driving style showed lower ($p < 0.05$) cortisol level than those transported under aggressive driving style. In conclusion, aggressive driving style cause acute stress in pigs, while bedding helps alleviate acute stress in pigs during transportation in LT.

Keywords (3 to 6): animal welfare, driving style, bedding, temperature, stress

Introduction

Animal welfare during transport became a substantial issue because of increasing demand for improved animal welfare standards [1]. The transportation of finishing pigs to the slaughterhouse is the final phase in pork production. Transport to the slaughterhouse is considered a complex and stressful event for pigs [2]. These stressful events can lead to mortality, skin damage and reduced meat quality, which can result in economic losses [3]. The stress factors associated with transportation have been studied so far, including loading and unloading, travel length, stocking density and climate conditions [4, 5].

In addition to the factors mentioned above, the welfare of pigs during transportation is impacted by vibration depending on the driver's driving style [6]. Poor driving techniques such as sudden acceleration, braking and cornering affect the ability of animals to maintain stable posture [7]. The vibration caused by vehicle motion during transportation leads to a displacement in a pig's center of gravity to be displaced in vertical, lateral, and horizontal directions resulting in discomfort and motion sickness for pigs [8]. A previous study showed that a good driving style is essential so that their bodies remain in the correct position with stress reduced to the minimum [9]. However, the International Organization for Standardization (ISO) does not report vibration levels that might be uncomfortable and potentially harmful to pigs. Bedding can help reduce transport losses as it is a significant component of the microenvironment [10]. Bedding materials such as foot battens, rubber mat can help pigs to avoid slipping and falling [11]. Grandin et al. [12] reported that bedding with deep straw can help to reduce frostbite. Several studies showed that bedding in the truck can help keep pigs warm and maintain their body temperature [13, 14]. However, most studies on the animal welfare during transportation have concentrated on the atmosphere and the temperature of the truck compartments [15, 16]. Our hypotheses are: (1) pigs are exposed to vibration forces, which can catalyze muscle fatigue and result in stress and behavior change during transportation; (2) bedding material might be a key element in managing the microclimate and enhancing the welfare of pigs while being transported. Thus, the objective of this study was to collect and quantify three axis acceleration and determine the effect of bedding during the transportation of pigs from farm to slaughterhouse.

Materials and Methods

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-1740-22-02).

Animals, pre-slaughter conditions and treatments

A total of 2,840 crossbred fattening pigs with a live weight of approximately 115 kg were used. They were raised in the same commercial farms and transported to the same commercial slaughterhouse. Farm and slaughterhouse were located in Korea and all experiments were conducted in 2022. Pigs were transported through 40 journeys of 40 km each that took approximately 1 hour. A 3×2×2 completely randomized factorial design was used to investigate effects of rubber-type bedding (bedding or non-bedding) and two levels of driving style (aggressive or normal) in three different time periods with different outside temperatures. Air temperature treatments were as follows: HT, high temperature (higher than 24°C); LT, low temperature (lower than 10°C); NT, normal temperature (10°C to 24°C).

Measurements

Acceleration measurements

Vibrations were collected along the x-axis in the direction of travel, horizontal vibrations along the y-axis, and vertical vibrations along the z-axis. The severity of the vibrations was measured using single-value estimates, which was used in this paper as the vibration dose value (VDV). To calculate VDV, acceleration data were filtered using a frequency weighting that put emphasis on frequencies known to be harmful to the human body to filter out those of less importance. The VDV for the x, y and z directions is given by:

$$VDV_{x,y,z} = \left[\frac{T_s}{N} \sum_{1}^{n=N} a_{x,y,z}^2 \right]^{0.25}$$

T_s denotes the measurement period, N the number of points and a_{x,y,z} is the frequency-weighted acceleration data in the x, y or z direction. The unit of VDV is m s^{-1.75}. The VDV_s were combined in the following formula to provide a global vibration level:

$$VDV_{total} = [VDV_x^4 + VDV_y^4 + VDV_z^4]^{0.25}$$

Measured accelerations during each transport were subdivided into intervals of 20 seconds, for each of which the VDV was calculated. These values were then averaged. Values of VDV_{total} were used to classify the driving styles (normal driving style, average VDV_{total} value: 8.5 m s^{-1.75}; aggressive driving style, average VDV_{total} value: 9.5 m s^{-1.75}).

Pork quality parameters measurements

According to Association of Official Analytical Chemists, the moisture, protein, and fat contents (%) were calculated [18]. After adding 50 mL of distilled water to 5 g of the sample from the left carcass loin, the pH was

determined. All samples were homogenized for 30 seconds using a homogenizer (Stomacher® 400 Circulator, Seward, UK), and their pH levels were determined using an Orion Star™ A211 pH Benchtop Meter (Thermo scientific™, USA) that was calibrated in phosphate buffer at pH 4, 7 and 10. With a Spectro Colorimeter (Model JX-777, Color Techno. System Co., Japan) calibrated on a white plate (L^* , 89.39; a^* , 0.13; b^* , -0.51), the meat color of a left carcass loin was measured. At this time, a white fluorescent lamp (D65) was used as the light source. L^* (lightness), a^* (redness) and b^* (yellowness) were used to represent color values. The test for the wetness of filter paper (FPW) was used to measure Drip loss (DL) [19]. Cooking loss (CL) was determined with Oliveira et al. [20] methodology. The CL value was calculated as the percentage change from the initial sample weight to the sample weight after heating. Five trained panelists rated the sensory color [21]. The sensory color was scored as follows: score 1 for pale, score 2 for grayish pink, score 3 for reddish pink, score 4 for purplish red, score 5 for dark. Five panelists assessed the marbling in accordance with the rigorous standards for evaluating animal products [22]. Marbling was scored as follow: score 1 (practically devoid), score 2 (slight), score 3 (modest), score 4 (slightly abundant), score 5 (abundant).

Pork quality classes measurements

For meat quality metrics, intra-measurement coefficients of variation were under 10%. According to parkunan et al. [23] (Table 1), pH values measured 45 m postmortem, DL variations, and light reflectance (L^*) were used to determine the different pork quality classes (pale, soft, and exudative, reddish-pink, soft, and exudative, red, firm, and nonexudative, and dark, firm, and dry).

Behavioral and status observations

On the ceiling of the trailer, cameras (Intelbras VMH 1010 D HD 720p, Intelbras SA, São José, Brazil) were used to continuously monitor behavior in real time. The quantity of pigs in each position (lying, standing, sitting, aggressive, and overlap; Table 2) was counted throughout travel. Only recordings with at least 7 clearly visible pigs in each group were utilized for the analysis since the compartment group was not always completely seen by the camera. Using only pigs that were captured on video for a minute, respiratory frequency was calculated as the number of breaths per minute. Using a thermal imaging camera (Xtherm, Xinfrared, China), skin temperature was taken 30 minutes before the commencement of transportation and 20 minutes after arrival during unloading. The temperature change before and after arrival was then analyzed.

Blood profile

Cortisol, lactate and glucose samples were taken after unloading at the lairage. Blood samples were collected from 10 pigs in each group for the determination of concentration levels of cortisol, creatine kinase, glucose and lactate. At least 3 mL of blood samples were taken from jugular vein. After collection, serum samples were centrifuged at 3000×g for 20 min at 4°C. Thereafter, the blood sample tubes were stored in a - 20°C refrigerator until analysis. The cortisol values in both transported and control pigs were measured using radioimmunoassay Coat-A-Count cortisol kits (Catalog number-TKCO5, Siemens Medical Solution Diagnostics, Los Anglos, CA, USA). Serum glucose was analyzed using an automatic Konelab analyser (Thermo Clinical Labsystems Oy, Vantaa, Finland) according to the manufacturer's instructions. Lactate levels were measured using a GM7 Analox analyser (Analox Instruments LTD, London, UK).

Statistical analysis

The experimental layout was a $3 \times 2 \times 2$ factorial arrangement. Data generated were subjected to a three-way ANOVA using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA). Significant differences ($P < 0.05$) were determined using Tukey multiple range test as included in the same statistical package. Pork quality classes proportion data were analyzed by the non-parametric Kruskal–Wallis test as the data were not normally distributed.

Results

Pork quality parameters measurements

Effects of driving style and bedding on pork composition and pork quality parameters during pre-slaughter pig transport are shown in Table 3. Pigs transported under aggressive driving style showed lower ($p < 0.05$) pH and WHC than those transported under normal driving style. Pigs transported under a normal driving style showed a lower ($p < 0.05$) percentage of DL ($p < 0.05$) than those transported under aggressive driving style. Pigs transported with bedding showed higher ($p < 0.05$) WHC and pH than those transported without bedding. Also, transported with bedding showed lower ($p < 0.05$) DL and L* value than those transported without bedding. Pigs transported in HT showed lower WHC ($p < 0.05$) than those transported in LT and NT. Pigs transported in LT showed lower ($p < 0.05$) pH than NT. A significant T×B interaction was observed for pH. Pigs transported with bedding showed higher ($p < 0.05$) pH than pigs transported without bedding in LT.

Behavioral and status observations and blood profile

pigs transported under aggressive driving groups showed a higher ($p < 0.05$) incidence of sitting behavior but lower ($p < 0.05$) incidence of lying behavior than those transported under normal driving style groups. Also, those transported with bedding showed lower ($p < 0.05$) sitting behavior but higher ($p < 0.05$) lying behavior than those transported without bedding. Pigs transported in HT showed lower ($p < 0.05$) sitting behavior but higher ($p < 0.05$) lying behavior than pigs transported in NT and LT. A significant T×B interaction was observed for lying behavior. Pigs transported with bedding showed higher lying behavior than pigs transported without bedding in LT. Pigs transported under normal driving style showed lower ($p < 0.05$) cortisol levels than those transported under aggressive driving conditions. Also, transported with bedding group showed lower ($p < 0.05$) cortisol levels than those transported without bedding.

Discussion

The process of transportation exposes pigs for the first time to various stressors throughout their lifetimes [24]. During transportation, vibrations and bedding are parameters that might impact pig welfare [25, 26]. In vehicles, animals are exposed to vibration and environmental variations, which can lead to physiological and behavioral disturbances [27]. Vibration, which is influenced by the state of the roads and the driving prowess of the driver, may jeopardize animal welfare [17]. Previous studies reported that vehicle vibration can be considered an acute stressor causing physiological and behavior stress in animals [28, 29]. Vibration exposure of pigs was compared with ISO thresholds for exposure action value (injury potential) of $8.5 \text{ m s}^{-1.75}$ for VDV [8]. Also, VDV of $9.1 \text{ m s}^{-1.75}$ has been established by the European Union [30]. However, in most studies, the exposure times to vibration was longer than those in our study [31, 32]. Considering the time exposed to vibration, our study classifies the driving styles (normal driving style, average VDV_{total} value: $8.5 \text{ m s}^{-1.75}$; aggressive driving style, average VDV_{total} value: $9.5 \text{ m s}^{-1.75}$). Pigs are exposed to high and low temperatures during transportation, depending on the weather conditions [33]. Heat and cold stress can both negatively impact pig mortality and welfare [34]. So, it is imperative to provide deep bedding to pigs during the winter in order to prevent frostbite [35].

In the current study, transported under aggressive driving style group showed lower WHC and pH than normal driving style group. According to Pérez et al. [36], pigs with acute stress before slaughter showed lower pH values than prolonged stress before slaughter. Due to its low pH and the resulting denaturation of myofibrillar and sarcoplasmic proteins, pale, soft and exudative (PSE) meat exhibits reduced WHC [37]. Meat quality may have been adversely affected due to physical stress caused by the necessity to stand at these locations due to the higher vibration [38]. According to Karunanayaka et al. [39], L^* value might be utilized to distinguish between pale samples and

normal samples with high sensitivity and high specificity. Also, lightness values can be used to evaluate the quality of the meat and assess the frequency of PSE conditions [40]. In our study, transported under aggressive driving style group showed the higher L* value and percentage of PSE pork than normal driving style group. This result is consistent with previous study that drops in pH is associated with increase L* value [41].

When pigs are subjected to acute stressors prior to slaughter, their concentration of muscle lactate increases and their muscle pH decreases, resulting in PSE pork [25]. Lower muscle pH is linked to decreased WHC, as observed in pale meat [42]. These result are consistent with our study that transported under aggressive driving style group showed higher percentage of PSE pork than transported with normal driving style group. It is often the case that pigs standing on a moving vehicle will fall or be trampled, resulting in bruised carcass [42, 44]. Our results can be explained that pigs exposed to thermal stress in LT and HT are acutely stressed by the aggressive driving style, resulting in adverse effects on pork quality. Also, bedding improve pork quality by reducing cold stress of pigs. During winter transport, pigs may suffer from cold stress and carcass bruising as a result of pigs standing or huddling to avoid contact with freezing truck aluminum surfaces [45]. Pasquale. [45] reported that pigs can retain heat and avoid frostbite when bedding is added to the floor of a trailer at a low temperature. Vermeer and Hopster. [46] reported that when pigs suffer from heat or cold stress, they can keep their temperature constant by adapting their behavior. According to Peeters et al. [17], pigs on a quiet journey stood at a lower proportion than those on a wild journey and laid at a higher proportion. Lying posture is considered as a resting behavior of pigs [47]. Hemsworth et al. [48] reported that aggression behavior is associated with stress. Our results can be explained that driving style affects aggressive behavior of pigs by giving acute stress on them. However, bedding can provide a warming effect, which can alleviate aggressive behavior in LT.

Cortisol, lactate and glucose levels in blood indicate how stress affects an animal's welfare and how stress level is created. Pigs that have experienced stress prior to slaughter demonstrate rapid glycolysis of muscle glycogen and increased production of lactate [6]. Cortisol is a hormone, which is synthesized by the adrenal cortex, a part of the adrenal gland. It plays a role in increasing blood flow, heart rate, and respiratory rate, leading to rapid pulses and breathing [49]. The cortisol level of pigs was found to increase under stress conditions, as reported in a previous study [50]. Our results can explain that why aggressive driving cause stress in pigs, which results in elevated cortisol level.

CONCLUSION

This study was conducted to investigate the possibility of reducing the transported pigs' stress levels by different driving style and bedding. Aggressive driving style has an effect on the accelerations of the truck and cause stress in

pigs. However, bedding can alleviate stress indicator such as aggressive behavior, cortisol, lactate and glucose in blood at low temperature. In conclusion, driving style led to transport stress in pigs, but pigs transported with bedding mitigate transport stress during transportation at low temperature.

Funding

This work was carried out with the support of “Cooperative Research program for Agriculture Science & Technology Development (Project No. PJ016214)” Rural Development Administration, Korea.

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Table 1. Determination of pork quality classes

Pork quality class	pH _{24h}	Drip loss (%)	L* value
PSE pork	< 6.0	≥ 5	≥ 50
RSE pork	< 6.0	≥ 5	42-50
RFN pork	< 6.0	2-5	42-50
PFN pork	< 6.0	2-5	≥ 50
DFD pork	≥ 6.0	≤ 2	< 42

PSE, pale, soft, exudative; RSE, reddish-pink, soft, exudative; RFN, red, firm, non-exudative; PFN, pale, firm, non-exudative; DFD, dark, firm, dry

Table 2. Description of the behaviors evaluated during transport

Behavior	Description
Basic behavior	
Standing	The act of standing still without any other action, with the forelimbs and hind legs stretched perpendicularly to the floor or similar behavior
Sitting	Two front legs straight to the floor, two rear legs and hips sitting in contact with the floor or similar behavior
Lying	The act of lying in the most comfortable position with the head, front legs, back legs, and abdomen touching the floor or similar behavior
Singularity behavior	
Aggression	Pushing, biting, or beating another pig with the head, lifting the pigs by pushing the head under the body or similar behavior
Overlap	The act of placing both forelimbs on the back of another pig or similar behavior

Table 3. Effects of driving style and bedding on pork composition and pork quality parameters during pre-slaughter pig transport in different temperature

Temperature (T)	< 10°C				10 to 24°C				> 24°C				SE	<i>P</i> - value					
Bedding (B)	-	+		-	+		-	+		D	B	T		D×B	D×T	T×B			
Driving style (D)	Nor	Agg	Nor	Agg	Nor	Agg	Nor	Agg	Nor								Agg		
Pork composition (%)																			
Moisture	73.93	73.43	74.18	73.95	74.14	74.34	74.12	74.27	73.93	74.09	73.83	73.87	0.281	0.852	0.717	0.180	0.912	0.342	0.364
Crude protein	22.27	22.72	22.34	22.65	21.89	21.84	21.74	21.25	22.77	23.04	22.79	22.35	0.264	0.960	0.123	0.542	0.166	0.203	0.554
Crude fat	2.62	2.25	2.63	2.79	3.16	2.71	3.00	3.28	2.57	2.42	2.63	2.83	0.205	0.618	0.247	0.112	0.325	0.882	0.971
Pork quality parameters																			
pH	5.49	5.37	5.56	5.50	5.66	5.53	5.65	5.55	5.54	5.48	5.60	5.44	0.027	<0.001	0.021	<0.001	0.994	0.807	0.020
WHC (%)	68.18	61.83	71.98	70.16	61.97	59.01	61.97	59.60	68.13	66.39	74.10	67.69	1.430	<0.001	<0.001	0.018	<0.001	0.723	0.928
DL (%)	4.51	4.95	3.90	4.20	4.44	5.33	4.48	5.14	3.40	4.10	3.27	3.60	0.187	<0.001	<0.001	0.077	0.207	0.309	0.252
CL (%)	25.08	24.29	23.26	25.72	22.08	24.57	21.91	24.60	25.70	25.94	24.44	26.02	0.815	0.091	0.546	0.894	0.245	0.231	0.092
L* value	51.26	52.75	48.67	49.50	50.17	51.32	50.26	51.10	49.30	50.18	46.53	49.70	0.798	0.003	0.001	0.143	0.316	0.620	0.633
a* value	7.12	6.73	6.91	7.34	7.19	7.03	7.18	6.93	8.38	6.80	7.60	7.86	0.438	0.078	0.710	0.914	0.266	0.535	0.093
b* value	5.27	5.56	5.25	5.45	6.39	6.49	6.32	6.66	5.22	5.03	5.19	4.97	0.305	0.102	0.907	0.958	0.603	0.500	0.920
Sensory evaluation																			
Sensory color ^a	2.85	2.70	3.54	2.85	3.00	2.53	3.07	2.80	3.12	2.80	3.16	2.87	0.193	0.522	0.056	0.389	0.001	0.917	0.633
Marbling ^b	3.43	3.17	3.12	3.18	2.73	2.60	3.13	2.73	3.42	2.82	3.16	3.08	0.219	0.018	0.745	0.398	0.064	0.729	0.440
Pork quality classes proportion (%)																			
PSE pork	20.0	26.7	6.7	13.3	0.0	6.7	0.0	0.0	6.7	13.3	6.7	13.3		0.215	0.321	0.027			

RSE pork	6.7	6.7	6.7	10.0	0.0	3.3	0.0	0.0	20.0	26.7	16.7	16.7	0.684	0.745	0.006
RFN pork	40.0	33.3	60.0	50.0	60.0	50.0	76.7	73.3	26.7	20.0	30.0	30.0	0.573	0.198	0.012
PFN pork	33.3	33.3	26.7	26.7	40.0	40.0	23.3	26.7	46.7	40.0	46.7	40.0	0.742	0.138	0.046
DFD pork	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000	1.000

Nor, normal (average VDV_{total} value: 8.5); Agg, aggressive (average VDV_{total} value: 9.5); -, without bedding; +, bedding; WHC, water holding capacity; DL, drip loss; CL, cooking loss

^aColor score ranged from 1 (pale color) to 5 (dark color)

^bMarbling score ranged from 1 (practically devoid) to 5 (abundant)

PSE, pale, soft, exudative; RSE, reddish-pink, soft, exudative; RFN, red, firm, non-exudative; PFN, pale, firm, non-exudative; DFD, dark, firm, dry

Table 4. Effects of driving style and bedding on behaviors, skin temperature and blood profile during pre-slaughter pig transport in different temperature

Temperature (T)	< 10°C				10 to 24°C				> 24°C				SE	P- value					
Bedding (B)	-		+		-		+		-		+								
Driving style (D)	Nor	Agg	Nor	Agg	Nor	Agg	Nor	Agg	Nor	Agg	Nor	Agg		D	B	T	D×B	D×T	T×B
Basic behavior, min/hour																			
Standing	50.61	49.60	52.33	51.97	50.53	50.20	50.17	50.53	49.60	51.33	48.60	52.13	0.756	0.136	0.143	0.281	0.233	0.006	0.079
Sitting	6.33	9.89	4.03	4.59	6.58	9.23	5.39	5.81	4.60	4.00	4.53	3.80	0.627	0.008	<0.001	<0.001	0.115	0.106	0.259
Lying	3.06	0.51	3.63	3.44	2.89	0.57	4.44	3.65	5.80	4.67	6.87	4.07	0.566	<0.001	<.0001	0.029	0.100	0.749	<0.001
Singularity behavior, count/hour																			
Aggression	5.94	8.93	6.47	5.80	4.73	5.47	5.27	4.33	5.27	4.60	6.13	5.27	0.657	0.087	0.463	0.087	0.795	0.111	0.016
Overlap	6.40	6.13	5.53	6.80	6.20	5.80	5.53	6.13	5.13	5.87	5.20	6.53	0.528	0.360	0.913	0.739	0.076	0.457	0.088
Respiratory frequency, count/min	63.11	63.00	62.43	60.33	61.50	59.93	61.20	59.67	68.07	65.53	64.67	64.87	1.234	0.068	0.064	0.572	0.075	0.963	0.855
Skin temperature (°C)																			
Before transport	37.45	37.33	37.39	37.41	37.41	37.37	37.40	37.50	37.39	37.40	37.41	37.51	0.076	0.766	0.298	0.841	0.780	0.584	0.163
After transport	39.43	39.54	39.45	39.56	39.54	39.71	39.69	39.62	39.49	39.57	39.68	39.76	0.104	0.108	0.186	0.414	0.186	0.937	0.508
Skin temperature change	1.99	2.21	2.07	2.15	2.13	2.34	2.29	2.12	2.10	2.17	2.27	2.25	0.083	0.127	0.472	0.400	0.158	0.424	0.136
Blood profile																			
Glucose, mg/dL	79.67	83.00	82.00	83.33	82.00	79.33	79.67	82.67	77.95	77.44	77.62	74.90	1.942	0.059	0.907	0.590	0.795	0.364	0.830
Lactic acid, mmol/L	3.52	6.03	3.23	4.29	2.35	6.28	2.28	4.19	3.97	5.05	4.27	5.52	0.548	0.070	0.081	0.116	0.082	0.087	0.090
Cortisol, ug/dL	3.33	6.15	2.02	3.64	2.48	4.80	1.27	3.17	5.05	4.92	4.93	4.69	0.602	<0.001	0.002	0.125	0.077	0.091	0.409

Nor, normal (average VDV_{total} value: 8.5); Agg, aggressive (average VDV_{total} value: 9.5); -, without bedding; +, bedding