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Abstract

There are several factors that affect the welfare and meat quality of pigs during pre-slaughter transport. Among various factors, the effects of weather conditions and loading density were studied. A total of 3,726 finishing pigs were allotted to one of nine groups arranged in a 3 × 3 factorial design according to the weather conditions (low temperature (LT), under 10℃; normal temperature (NT), 10-24℃; high temperature (HT), upper 24℃), and loading density (low density (LD), upper 0.43 m2/100 kg; normal density (ND), 0.37-0.43 m2/100 kg; high density (HD), under 0.37 m2/100 kg). Each treatment group follow as: LTLD, LTND, LTHD, NTLD, NTND, NTHD, HTLD, HTND, HTHD. In terms of carcass composition, pigs had the highest carcass weight and backfat thickness at LT. Comparing the HD transport to the ND transport, the meat quality indicated a lower pH and more drip loss. The incidence rate of pale, soft, exudative (PSE) pork was high in the order of the HD, LD, and the ND transport (20%, 9%, and 2%, respectively). The HT transport showed the lowest pH and greatest L* value under the given weather conditions. Pigs transported under the HTHD and LTLD conditions had the greatest rates of PSE pork (40% and 20%, respectively). Pigs exposed to HD transport had the shortest laying time and the highest overplap behavior. The LDLT transport pigs had a shorter laying time than the LDNT and LDHT transport pigs. In conclusion, too high or too low density transport is generally not excellent for meat quality or animal welfare, however it is preferable to transport at a slightly low density at high temperature and at a slightly high density at low temperature.

Keywords : Transport, loading density, temperature, welfare, meat quality

Introduction

Animal welfare for farm animals has become a major issue in the livestock industry in recent years. Urbanization, the media, the influence of civil society groups, and the rise of society's educational and economic standards have made people question how and under what conditions food is brought to the table from the farm [1]. The process from farm to table can be classified into three stages from an animal welfare point of view on pigs: i) raising, ii) transportation, iii) pre-slaughter and slaughter. Among them, many studies have been conducted on the transportation because it not only poses a strong stress to pigs in the shortest time, but also causes enormous economic loss through damage to meat quality [2, 3].

Factors such as driving, road quality, duration of transport, stocking density, floor surface and bedding, and climatic conditions like air temperature can cause transport stress to pigs [4]. Stress reactions overtax the body systems and cause reduction in fitness of the animal by inducing dysfunctions of the pituitary, adrenal and thyroid glands, resulting in carcass depreciation and meat quality defects [5, 6]. Extreme ambient temperatures during journey are regarded as one of the most significant contributing factors for heat stress and increase in loss rates [7, 8]

Pigs are homeothermic animals and have limited thermoregulatory ability, with minimal functional sweat glands, meaning they are very sensitive to thermal stress [9-11]. Pigs exposed to temperatures beyond the thermal comfort zone (TCZ) will become stressed. Their glycolysis will accelerate and muscle pH will fall rapidly [1]. As the pH of the muscle drops sharply and the slaughter temperature of the muscle approaches body temperature, some filaments (myosin) are denatured [12]. Meat with deteriorated myosin structure leaks and water activity increases, resulting in increased microbial growth and low quality such as pale, soft exudative (PSE) meat [13]. Also, higher prevalence of dark, firm, and dry (DFD) meat has been reported when pigs are exposed to temperature below the TCZ [14].

During transport, pigs must have sufficient space to stand and lie freely in its natural position without risk of injury or suffering [15]. Optimal loading densities for pigs during transport require a compromise between economic concerns of requiring the highest possible loading densities to reduce the burden of transport costs and the concerns of animal welfare [16]. In 2004, EU requirement was 235 kg/m^2 space for 100 kg pigs during transport [17]. However, for countries like Korea with a large amount of pig production per area can cause a short transport time around one hour. Thus, the EU's 8-hour standard is not suitable. In addition, effects of the interaction between stocking density and air temperature on animal welfare parameters and carcass and meat quality of pigs have not been reported yet [18].

Therefore, the aim of this study was to investigate effects of air temperature and loading density during transportation for a short period of time (less than 2 hours) on the welfare, carcass, and meat quality of pigs.

Materials and Methods

Ethics

The experimental protocol was approved (CBNUA-2035-22-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

Animals, pre-slaughter conditions and treatments

A total of 3,903 crossbred pigs of mixed sex with same genetics ([Yorkshire × Landrace] × Duroc) were transported from the one commercial finishing farms to the one commercial slaughterhouse. Firm and slaughterhouse were located in Korea. At the moment of loading, the animals had been deprived of food for 12 h. The experiment was conducted for one year in 2021. Pigs were transported through 59 journeys with travelling a distance of 40 km. Travel conditions and handling were the same for all pigs. Animals were always herded using pig boards and without using sticks or electrical goads. Transport density was set with reference to animal welfare regulations in Korea, Europe, and the United States, and temperature was set in consideration of the four seasons in Korea, mainly transported between 6:00 and 12:00 [15, 19, 20]. Density treatments were as follows: LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg); HD, high density (higher than 0.37 m^2/100 kg). Air temperature treatments were as follows: LT, low air temperature (lower than 10℃); NT, normal temperature (10℃ to 24℃); HT, high temperature (higher than 24℃) This design was proposed emphasizing the control of all the factors associated the experimental treatment (genotype, fasting, handling, bedding, distance, and lairage) in order to compare only the effect of transport density and air temperature.

Carcass quality measurements

Pig carcasses were graded with the Korean Pig Carcass Grade System [21] (Figure 1). The conductor grades are as follows: 1+ grade (carcass weight: 83 to 93 kg, backfat thickness: 17 to 25 mm), 1 grade (carcass weight: 80 to 98 kg, backfat thickness: 15 to 28 mm, the rest except for 1+ grade), 2 grade (Ranges of carcass weight and backfat thickness that do not correspond to 1, 1+ grade). The hot carcass weight was measured on an electronic scale 45 minutes postmortem and expressed in integer kg units. The left half carcass was used to measure the backfat thickness. The backfat thickness between the last thoracic vertebra and the first lumbar vertebra and that between the 11^{th} and 12^{th} thoracic vertebrae were measured with a ruler. Hot carcass weight and backfat thickness were measured and calculated as [backfat thickness (mm) / hot carcass weight (kg)]. Pig losses were measured by observing and classifying fractures and bruises after the pigs were unloaded after transport.

Pork quality parameters measurements

The moisture, protein, and fat content (%) was determined according to Association of Official Analytical Chemists (AOAC) [22]. The pH was measured after adding 50 mL of distilled water to 5 g of the left carcass loin. All samples were homogenized for 30 seconds using a homogenizer (Stomacher® 400 Circulator, Seward, UK), and then measured with a pH meter (Orion Star™ A211 pH Benchtop Meter, Thermo scientific™, USA) calibrated in phosphate buffer at pH 4, 7 and 10. In meat color, left carcass loin was measured with a Spectro Colorimeter (Model JX-777, Color Techno. System Co., Japan) standardized on a white plate (L*, 89.39; a*, 0.13; b*, -0.51). At this time, the light source was used a white fluorescent lamp (D65). Color values were expressed as L*(lightness), a*(redness), b*(yellowness). Drip loss (DL) was assessed using the filter paper wetness (FPW) test [23]. Cooking loss (CL) was determined with Oliveira et al. [24] methodology. CL value was measured as the ratio (%) of the weight of the initial sample to the weight after heating the sample. Sensory color was evaluated by 5 trained panelists [25]. The sensory color was followed as: score 1 (pale), score 2 (grayish pink), score 3 (reddish pink), score 4 (purplish red), score 5 (dark). Marbling was evaluated by 5 panelists according to the detailed criteria for grading of livestock products [26] (Figure 2). Marbling score was followed as: score 1 (practically devoid), score 2 (slight), score 3 (modest), score 4 (slightly abundant), score 5 (abundant).

Pork quality classes measurements

The intra-measurement coefficients of variation for meat quality parameters were below 10%. Pork quality classes (pale, soft, and exudative - PSE; red, soft, and exudative -RSE; red, firm, and nonexudative - RFN; pale, firm, and nonexudative - PFN; dark, firm, and dry - DFD) were determined using pH values measured 24 h postmortem, DL variations, and light reflectance (L*), according to Koćwin-Podsiadła et al. [27] (Table 1).

Behavioral and physiological parameters

During transport, behaviors were continuously recorded using cameras (Intelbras VMH 1010 D HD 720p, Intelbras SA, São José, Brazil), installed on the ceiling of the trailer. During transport, the number of pigs in each posture (lying, standing, sitting, aggression, and overlap; Table 2) was recorded. As the compartment group was not always entirely visible by the camera, only recordings with at least 7 visible pigs in each group were used for the analysis. Respiratory frequency measured the number of breaths per minute using only pigs observed by the camera for 1 minute. Changes in skin temperature were measured at a distance of 1 m 30 minutes before the start of transportation and 20 minutes after arrival during unloading through a thermal imaging camera capable of measuring long-wavelength infrared (Xtherm, Xinfrared, China). The thermal imaging camera has infrared resolution of 1920 pixels (160 × 120), visual resolution of 1440 × 1080, emissivity of 95%, and was used after sufficient calibration for accurate measurement with an accuracy of ± 3℃.

Statistical analysis

The experimental layout was a 3 × 3 factorial arrangement. Data generated were subjected to a two-way Analysis of Variance using SAS software (Statistical Analysis System Software, 2012). Statistics for each factor were analyzed using general linear model (GLM) procedures of SAS.

Significantly ($p < 0.05$) different means among the variables were separated using tukey multiple range test.

Results and discussion

Bringing pigs from farm to table necessarily involves transportation of pigs to the slaughterhouse. As pigs are transported, several human-animal interactions and environmental factors can affect pig welfare [28]. Positive and negative effects of such factors on animal welfare during transportation can be measured using behavioral, physiological, and carcass and meat quality parameters [29]. The present study provides an overview of the effects of air temperature and loading density during transport for a short period of time on the welfare, carcass, and meat quality of pigs in Korea.

Effects of loading density on carcass composition and carcass grade during pre-slaughter pig transport are shown in Table 3. Loading density during pre-slaughter pig transport did not significantly ($p > 0.05$) affect carcass composition traits or carcass grade. Therefore, it is considered that transport density does not affect carcass weight and backfat thickness in transport for less than 3 hours. Previous studies reported similar results that transport density did not affect carcass weight and backfact thickness in transport for less than 3 hours [30-32]. Therefore, it is considered that transport density does not affect carcass weight and backfat thickness in transport for less than 3 hours.

Effects of air temperature on carcass composition and carcass grade during pre-slaughter pig transport are shown in Table 4. LT transport group had higher ($p < 0.05$) hot carcass weight, back fat thickness, and backfat thickness/hot carcass weight ratio compared to NT and HT transport groups. The NT transport group had lower ($p < 0.05$) backfat thickness and backfat thickness/hot carcass weight ratio compared to LT and HT transport groups. The lowest ($p < 0.05$) carcass grade score was recorded in the HT transport group. Similar to this result, Čobanović et al. [33] have reported that pigs slaughtered in summer show lower hot carcass weight and backfat thickness compared to pigs slaughtered in winter. Čobanović et al. [30] also reported that pigs slaughtered in winter had the highest slaughter weight and backfat thickness. These results are probably influenced by the season during the fattening process in pig houses. Hale [34] and Goumon et al. [35] reported that pigs fattened in winter had a higher carcass weight and backfat thickness because they intake more feed than in summer. To reduce heat production associated with digestion and metabolism of nutrients, heat-stressed pigs reduced feed intake [36]. Also, in carcass grade, the HT transport showed lower grade 1+, grade 1 rate and higher grade 2 rate compared the NT and the LT transport. Although hot carcass weight and back fat thickness were similar to those of NT transport, the significantly lower carcass grade score means that pigs raised at high temperatures did not have uniform carcass characteristics.

Interactive effects of air temperature and loading density on carcass composition and carcass grade during pre-slaughter pig transport are shown in Table 5. The effect of the interaction of air temperature and loading density did not show a significant difference. This indicated that pork composition and pork quality parameters were only affected by air temperature.

Effects of loading density on pork composition and pork quality parameters during pre-slaughter pig transport are shown in Table 6. Loading density had no significant ($p > 0.05$) effect on content of moisture, crude protein, or crude fat. However, regarding pork quality parameters, the ND transport group had higher ($p < 0.05$) pH but lower ($p < 0.05$) DL and L* value than LD and HD transport groups. The LD transport group had lower ($p < 0.05$) DL and L* value than the HD transport group. Contrary to these results, Warriss et al. [37] have reported that loading densities (0.50, 0.41, 0.36, and 0.31 m^2/100 kg) do not affect meat quality. Urrea et al. [38] have also reported that pH, DL, and L*, a*, b* values of loin muscles show no difference at different loading densities (0.50, 0.43, and 0.37 m^2/100 kg). However, Driessen et al. [39] have reported that lower density is related to a higher pH of loin muscle. Carr et al. [40] have also reported a higher DL in meat quality during short transportation time at high loading density. These conflicting results might be due to different stress factors (transportation time, pig breed, sex, driving style, bedding presence, and so on) of pigs. A possible explanation to understand findings of this study is that densities higher or lower than 0.37 m^2/kg to 0.43 m^2/kg give pigs a more stressful situation and cause depletion of muscle glycogen, which in turn leads to the production of lactic acid in the muscle that can reduce the pH [41]. This might be related to the stress of pigs in a too large or too small space. The higher DL in HD and LD transport groups than in the ND transport group might be due to muscle pH value. The high internal lactic acid concentration can change electrostatic charge to decrease the volume of myofibrils in the cell, which reduces protein solubility of myoplasm and myofibrils, thereby lowering water holding capacity (WHC) of muscles and increasing the DL [42]. Regarding pork quality, the ND transport group showed lower probability of PSE pork occurrence but higher probability of RFN pork occurrence than LD and HD transport groups. Similar to the results of this study, Pereira et al. [43] have reported difference RFN appearance rates according to loading density. At loading densities of 0.42 m^2/100 kg, 0.40 m^2/100 kg, and 0.36 m^2/100 kg, RFN pork appearance rates were 50%, 53%, and 21%, respectively. Čobanović et al. [44] have also reported that the transport density of 0.3-0.50 m^2/100 kg has lower incidence of PSE than transport density higher or lower than 0.3-0.5 m^2/100 kg. The EU recommends the minimum space allowance for pigs is 0.425 m^2/100 kg. However, previous studies have shown that the application of EU requirement for loading density should be adjusted according to transport time [45]. Guàrdia et al. [46] have reported that loading density higher than 0.50 m^2/100 kg can decrease the incidence of PSE pork compared to a loading density of 0.5 m^2/100 kg during short journeys of about 1 hour. Cussen and Garces [47] have also recommended a density of 0.36 m^2/100 kg for short transport and lower than 0.36 m^2/100 kg for long transport. In general, scientific evidence suggests that loading density lower than 0.43 m^2/kg with a short transport (less than 2 hours) has an adverse effect on pork quality.

Effects of air temperature on pork composition and pork quality parameters during pre-slaughter pig transport are shown in Table 7. Regarding pork compositions, the NT transport group had higher (p < 0.05) crude protein content but lower (p < 0.05) crude fat content than LT and HT transport groups. As for pork quality parameters, the HT transport group had lower (p < 0.05) pH, WHC, and sensory color, but higher (p < 0.05) DL, CL, L* value, and b* value than LT and HT transport groups. In this study, the HT transport group showed higher L* value, b* value, and DL than LT and NT transport groups. Also, the HT transport group had a lower pH of pork than LT and NT transport groups. Low pH, high L* value, and high DL of pork are indicators of increased probability of PSE meat. Cruzen et al. [48] have reported that heat stress of about 2 hours has a measurable effect on muscle protein, impairing muscle structure, function, and pork quality. Similar to this results, previous studies have also reported that high temperature has a harmful effect on pork quality [49-52]. In general, the higher the muscle temperature, the higher the lactic acid production after slaughter [53-56]. Under normal circumstances, after slaughter, muscle pH declines slowly over a 6–8 hour period before the onset of post-mortem rigidity [57]. However, under abnormal circumstances such as acute stress before slaughter, adrenergic mechanisms can increase muscle glycogenolysis and result in increased muscle temperature, leading to steep decrease of muscle pH [58]. Muscle pH is a key factor affecting muscle WHC and color of fresh pork [59]. WHC increases as muscle pH moves away from the isoelectric point (5.0 to 5.1) [60]. The reason is that a sudden decrease in pH causes denaturation of myosin, which denatures proteins, thereby blocking the polar group and reducing the WHC [60, 61]. Also, a drop in pH is usually associated with an increase in L* value indicative of PSE pigs [62]. Previous studies have reported a negative relationship between L* and pH [63]. In conclusion, the frequency of PSE pork was low in the order of NT, LT, and HT, whereas the frequency of RFN pork was high. Previous studies have also reported that an increase of air temperature can lead to higher incidence of PSE pork [64-67]. These results show that the probability of PSE pork occurrence is the lowest when pigs are transported at a thermal comfort zone temperature and that heat stress can increase the probability of PSE pork occurrence compared to cold stress.

Interactive effects of air temperature and loading density on pork compositions and pork quality parameters during pre-slaughter pig transport are shown in Table 8. Two-way interaction between air temperature and loading density affected ($p < 0.05$) pork composition, pH, WHC, DL, CL, L*, a*, and b* value. Pigs exposed to high loading density in high temperature produced meat with the lowest pH, WHC, and a* value but the highest DL, CL, and a* value. These results are explained by Pereira et al. [43] who reported that high-density pig transport restricts airflow between pigs caused reducing heat loss and increasing the air temperature inside of truck compared to outside. The narrow, hot and unfriendly transport environment increases heat stress and consequently promotes muscle metabolism, which increases lactic acid formation in skeletal muscle [33]. This results in a rapid decrease in pH in the early post-mortem muscle, resulting in denaturation of sarcoplasmic and myofibrillar proteins, and finally the generation of PSE pork with poor water holding capacity [62, 68, 69]. In addition, in the results of this study, high-density transportation at high temperature increased the incidence of PSE meat the most compared to other treatments.

Behavioral responses such aggression in pigs are clear indicators of animal welfare status [29, 70]. However, behavioral responses of pigs during transport and their effects on the quality of pork consumption have not been extensively investigated worldwide [28]. Pig behaviors such as sitting, lying down, aggression, overlap and pig fighting during transport can be recorded with a video recorder and consequently assessed in relation to animal welfare and meat quality [28]. During transport, pigs may become depressed from bruises or injuries, which may result in the release of cortisol, vasopressin, epinephrine, creatinine kinase, lactate dehydrogenase and norepinephrine into the bloodstream [29]. These hormones can breakdown the stored glycogen inside muscles and fat, causing low quality of pork [71]. Therefore, suitable transport conditions are needed to reduce aggressive behavior and provide a comfortable situation for pigs.

Effects of loading density on pig behaviors, skin temperature, and respiratory frequency during pre-slaughter pig transport are shown in Table 9. Regarding basic behavior, the HD transport group had higher ($p < 0.05$) sitting time but lower ($p < 0.05$) lying time than LD and ND transport groups. The HD transport group also showed higher ($p < 0.05$) overlap behavior than ND and LD transport groups. Regarding aggression behavior and respiratory frequency, the ND transport group showed lower ($p < 0.05$) rates than LD and the HD transport groups. The skin temperature difference before and after transport was higher ($p < 0.05$) in the HD transport group than in LD and ND transport groups. In this study, the lying time during the transport was less than 5%. There results were in agreement with previous reports showing that few pigs lied down during a short transport [72-74]. Among them, a density higher than 0.37 m^2/100 kg resulted in a significantly lower lying time than a lower density. These results indicate that pigs feel uncomfortable for take a stance when the density is higher than 0.37 m^2/100 kg, which leads to an increase in singularity behavior. For overlap behavior, similar to our results, Guise and Penny [75] reported that the frequency of mounting (overlap) behavior increased linearly as the loading density increased (0.50 m^2/100 kg, 0.38 m^2/100 kg, and 0.33 m^2/100 kg) during transport. Bracke et al. [9] have also reported that if pigs lie on top of each other (overlap), it could be a sign of a high stock density.

However, in aggression behavior, LD and HD transport groups showed higher frequency than the ND transport group. Pigs cannot support each other when the truck has a large floor space. Therefore, pigs have difficulty maintaining their standing balance when trucks are accelerating, braking, and rotating [11]. These results indicated that providing more transport space does not result in more pigs lying down, leading to more aggression as animals have difficulty balancing.

Effects of air temperature on pig behaviors, skin temperature, and respiratory frequency during pre-slaughter pig transport are shown in Table 10. In basic behavior, the LT transport group had higher ($p < 0.05$) standing time but lower ($p < 0.05$) lying time rate than NT and HT transport groups. The HT transport group showed higher ($p < 0.05$) lying time than LT and NT transport groups. In singularity behavior, the NT transport group showed lower ($p < 0.05$) aggression behavior than LT and the HT transport group and the LT transport group showed higher ($p < 0.05$) overlap behavior than the NT transport group. The HT transport group showed higher ($p < 0.05$) respiratory frequency and skin temperature change than LT and NT transport groups. In this study, pigs also showed increased lying time as temperature increased. Similarly, Torrey et al. [76] have reported that pigs transported during summer show higher lying time than pigs transported during winter. Lying down behavior is often used as a diagnostic tool to assess thermal conditions [77, 78]. In cold temperature, pigs are posed to reduce surface area attached to the floor to minimize heat loss [79]. Conversely, in hot temperature, pigs tend to lie down to increase heat loss [35]. Čobanović et al. [33] have reported that both heat and cold stress could provoke fighting behavior in pigs. This finding is further supported by the finding that the highest levels of stress enzymes creatine kinase and lactate dehydrogenase are recorded in pigs slaughtered in winter and summer [14, 80]. Also, under cold stress conditions, pigs exhibit huddling (overlap) behavior to create a warmer climate and conserve body energy, increasing their ability to withstand cold temperatures during transport [50, 81].

Interactive effects of air temperature and loading density on pig behaviors, skin temperature, and respiratory frequency during pre-slaughter pig transport are shown in Table 11. Two-way interaction between air temperature and loading density affected ($p < 0.05$) pig behaviors (standing time rate, lying time rate) and skin temperature change. As the temperature rises, most pigs begin to lie down to maximize heat loss through contact with truck floors or walls, especially in hot weather conditions due to heat exhaustion [45, 82]. Compared to pigs under high and normal loading density conditions, those exposed to a high loading density showed no significant difference in lying time or standing time. These results indicate that high loading density (space for pigs lower than 0.370 m^2/100 kg) might cause pigs not to lie down in its natural position during transportation. Also, in this study, two-way interaction between air temperature and loading density affected ($p < 0.05$) pig behavior (aggression behavior frequency) and skin temperature change. The highest aggression behavior frequency and skin temperature change were recorded for pigs exposed to a high loading density in a high air temperature. When the environmental temperature exceeds the TCZ, pig begins to find a cool place to lie down without contacting other pigs [83]. In an environment that cannot lie down, pigs become agitated, increasing aggression between groups [83]. Therefore, pigs subjected to a high air temperature with a high loading density probably experienced critical acute stress caused by narrow space that could not allow each pig to lie down to radiate heat out of the body. In contrast, the LD transport group showed higher ($p < 0.05$) aggression behavior frequency at low air temperature than at normal and high air temperatures. It can be argued that a loading space of at least 0.370 m^2/100 kg is needed for pre-slaughter pigs to have better transport welfare during a high air temperature (upper 24℃). At lower temperatures, it is recommended to transport pigs at a density higher than 0.430 m^2/100 kg.

CONCLUSION

Based on obtained results, transport of too high (higher than 0.37 m^2/100 kg) or low (lower than 0.43 m^2/100 kg) density is generally not good for meat quality and animal welfare, but it is desirable to transport at a slightly lower density at high temperatures and at a higher density at low temperatures.

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Tables and figures

Table 1. Determination of pork quality classes [21]

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, red, sift, 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 loading density on carcass composition and carcass grade during pre-slaughter pig transport

	LD	ND	HD	SEM	P-value
N	1073	1737	1093	-	-
Carcass composition traits					
Hot carcass weight (kg)	84.9	85.16	84.87	0.09	0.320
Backfat thickness (mm)	19.55	19.34	19.62	0.07	0.162
Backfat thickness/ hot carcass weight ratio (mm/kg)	0.230	0.227	0.261	0.001	0.092
Carcass grade					
Grade 1+ (%)	40.7	38.8	37.9	-	-
Grade 1 (%)	34.7	35.9	32.9	-	-
Grade 2 (%)	24.6	25.3	29.2	-	-
Carcass grade score[1)]	2.160	2.134	2.093	0.013	0.142
Pig losses					
Fracture (n)	3	1	1	-	-
Bruises (n)	2	0	1	-	-

[1)] Carcass grade score was determined as follows: 3, grade 1+; 2, grade 1; 1, grade 2

LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg loading density); HD, high density (higher than 0.37 m^2/100 kg)

Table 4. Effects of air temperature on carcass composition and carcass grade during pre-slaughter pig transport

	LT	NT	HT	SEM	P-value
N	2156	1196	551	-	-
Carcass composition traits					
Hot carcass weight (kg)	85.90[a]	84.05[b]	83.84[b]	0.09	<0.001
Backfat thickness (mm)	20.16[a]	18.36[c]	19.28[b]	0.68	<0.001
Backfat thickness/ hot carcass weight ratio (mm/kg)	0.234[a]	0.218[c]	0.229[b]	0.001	<0.001
Carcass grade					
Grade 1+ (%)	40.1	40.2	33.0	-	-
Grade 1 (%)	37.4	32.6	29.2	-	-
Grade 2 (%)	22.5	27.2	37.8	-	-
Carcass grade score[1)]	2.176[a]	2.131[a]	1.953[b]	0.013	<0.001
Pig losses					
Fracture (n)	2	1	2	-	-
Bruises (n)	0	2	1	-	-

[a-c]Means in the same row with different superscripts differ ($p < 0.05$)

[1)] Carcass grade score was determined as follows: 3, grade 1+; 2, grade 1; 1, grade 2

LT, low air temperature (lower than 10℃); NT, normal temperature (10℃ to 24℃); HT, high temperature (higher than 24℃)

Table 5. Effects of interaction between loading density and air temperature on carcass composition and carcass grade during pre-slaughter pig transport

	LT			NT			HT			SEM	P-value	
	LD	ND	HD	LD	ND	HD	LD	ND	HD		Treatments	Interaction
N	659	921	576	291	647	258	123	169	259	-	-	-
Carcass composition traits												
Hot carcass weight (kg)	85.82^{ab}	86.26^{a}	85.30^{abc}	83.30^{d}	84.08^{cd}	84.58^{bcd}	83.81^{d}	83.30^{d}	84.21^{cd}	0.09	<0.001	0.072
Backfat thickness (mm)	20.29^{a}	20.03^{ab}	20.17^{ab}	17.98^{d}	18.38^{cd}	18.68^{cd}	19.26^{abc}	19.18^{bc}	19.35^{abc}	0.07	<0.001	0.318
Backfat thickness hot carcass weight ratio (mm/kg)	0.236^{a}	0.232^{a}	0.236^{a}	0.216^{d}	0.218^{cd}	0.220^{bcd}	0.229^{abc}	0.230^{ab}	0.229^{abc}	0.001	<0.001	0.323
Carcass grade												
Grade 1+ (%)	43.4	38.8	38.5	39.9	41.3	36.8	28.5	29.6	37.4	-	-	-
Grade 1 (%)	35.4	38.8	37.7	33.3	33.4	29.1	34.1	30.2	26.3	-	-	-
Grade 2 (%)	21.2	22.4	23.8	26.8	25.3	34.1	37.4	40.2	36.3	-	-	-
Carcass grade score[1)]	2.220^{a}	2.163^{ab}	2.148^{ab}	2.131^{ab}	2.156^{ab}	2.054^{abc}	1.911^{c}	1.894^{c}	2.012^{bc}	0.013	<0.001	0.124
Pig losses												
Fracture (n)	1	0	1	0	1	0	2	0	0	-	-	-
Bruiser (n)	0	0	0	2	0	0	0	0	1	-	-	-

$^{a\text{-}d}$Means in the same row with different superscripts differ ($p < 0.05$)

[1)]Carcass grade score was determined as follows: 3, grade 1+; 2, grade 1; 1, grade 2

LT, low air temperature (lower than 10℃); NT, normal temperature(10℃ to 24℃); HT, high temperature(higher than 24℃)

LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg); HD, high density (higher than 0.37 m^2/100 kg)

Table 6. Effects of loading density on pork composition and pork quality parameters during pre-slaughter pig transport

	LD	ND	HD	SE	P-value
Pork composition (%)					
Moisture	73.86	74.29	74.06	0.09	0.134
Crude protein	22.08	21.78	22.18	0.09	0.161
Crude fat	2.80	2.55	2.64	0.08	0.456
Pork quality parameters					
pH	5.51^{b}	5.57^{a}	5.51^{b}	0.01	0.036
WHC (%)	64.46^{ab}	67.12^{a}	61.19^{b}	0.67	0.001
DL (%)	4.32^{b}	3.62^{c}	5.10^{a}	0.11	<0.001
CL (%)	25.09^{b}	24.15^{b}	29.32^{a}	0.44	<0.001
L* value	50.93^{b}	48.10^{c}	53.93^{a}	0.48	<0.001
a* value	7.24^{ab}	7.83^{a}	6.53^{b}	0.16	0.003
b* value	5.27	5.37	5.57	0.15	0.722
Sensory color[1)]	3.09	3.04	2.76	0.06	0.077
Marbling[2)]	3.18	3.15	2.97	0.07	0.412
Pork quality classes (%)					
PSE pork	8.8	2.2	20.0	-	-
RSE pork	8.8	0.0	17.8	-	-
RFN pork	37.9	80	31.1	-	-
PFN pork	44.5	17.8	31.1	-	-
DFD pork	0.0	0.0	0.0	-	-

$^{a-c}$Means in the same row with different superscripts differ ($p < 0.05$)

LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg); HD, high density (higher than 0.37 m^2/100 kg)

WHC, water holding capacity; DL, drip loss; CL, cooking loss

[1)]Color score ranged from 1 (pale color) to 5 (dark color)

[2)]Marbling score ranged from 1 (practically devoid) to 5 (abundant)

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

Table 7. Effects of air temperature on pork composition and pork quality parameters during pre-slaughter pig transport

	LT	NT	HT	SE	P-value
Pork composition (%)					
Moisture	73.79	74.17	74.26	0.09	0.074
Crude protein	21.63[b]	22.49[a]	21.93[b]	0.09	<0.001
Crude fat	3.11[a]	2.07[b]	2.82[a]	0.08	<0.001
Pork quality parameters					
pH	5.52[a]	5.57[a]	5.51[b]	0.01	0.470
WHC (%)	63.39[b]	69.65[a]	59.73[c]	0.67	<0.001
DL (%)	3.92[b]	4.20[b]	4.91[a]	0.11	0.001
CL (%)	25.54[b]	24.89[b]	28.13[a]	0.44	0.005
L* value	50.25[b]	49.31[b]	53.39[a]	0.48	0.001
a* value	7.07	7.19	7.35	0.16	0.778
b* value	5.06[b]	4.38[b]	6.77[a]	0.15	<0.001
Sensory color[1)]	3.06[a]	3.12[a]	2.70[b]	0.06	0.012
Marbling[2)]	3.42[a]	2.64[b]	3.24[a]	0.07	<0.001
Pork quality classes (%)					
PSE pork	6.7	4.4	15.6	-	-
RSE pork	2.2	11.1	11.1	-	-
RFN pork	57.8	60	37.8	-	-
PFN pork	33.3	24.5	37.5	-	-
DFD pork	0.0	0.0	0.0	-	-

[a-c]Means in the same row with different superscripts differ ($p < 0.05$)

LT, low air temperature (lower than 10℃); NT, normal temperature(10℃ to 24℃); HT, high temperature(higher than 24℃)

[1)]Color score ranged from 1 (pale color) to 5 (dark color)

[2)]Marbling score ranged from 1 (practically devoid) to 5 (abundant)

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

Table 8. Effects of interaction between stocking density and air temperature on carcass composition and carcass grade during pre-slaughter pig transport

	LT			NT			HT			SE	P-value	
	LD	ND	HD	LD	ND	HD	LD	ND	HD		Treatments	Interaction
Pork composition (%)												
Moisture	73.37^{b}	73.97^{ab}	74.04^{ab}	73.43^{b}	74.88^{a}	74.18^{ab}	74.79^{a}	74.03^{ab}	73.95^{ab}	0.24	<0.001	<0.001
Crude protein	21.67^{bc}	21.16^{c}	22.05^{abc}	22.45^{ab}	22.14^{abc}	22.87^{a}	22.11^{abc}	22.04^{abc}	21.63^{bc}	0.09	<0.001	0.074
Crude fat	3.12^{abc}	3.29^{ab}	2.92^{abc}	2.81^{bcd}	2.05^{de}	1.35^{e}	2.48^{cd}	2.32^{cd}	3.66^{a}	0.08	<0.001	<0.001
Pork quality parameters												
pH	5.45^{bc}	5.54^{ab}	5.58^{ab}	5.50^{bc}	5.65^{a}	5.56^{ab}	5.58^{ab}	5.53^{abc}	5.41^{c}	0.01	<0.001	<0.001
WHC (%)	61.17^{cd}	65.55^{bc}	61.45^{cd}	71.06^{ab}	75.02^{a}	62.86^{cd}	61.14^{cd}	58.80^{d}	59.25^{d}	0.67	<0.001	<0.001
DL (%)	4.73^{b}	3.49^{cd}	3.54^{cd}	4.57^{b}	3.18^{d}	4.86^{b}	3.68^{cd}	4.18^{bc}	6.89^{a}	0.11	<0.001	<0.001
CL (%)	27.66^{bc}	26.19^{c}	22.77^{d}	26.49^{c}	19.21^{e}	28.96^{b}	21.12^{d}e	27.05^{bc}	36.23^{a}	0.44	<0.001	<0.001
L* value	53.24^{b}	46.73^{c}	50.78^{bc}	49.73^{bc}	46.96^{c}	51.24^{bc}	49.81^{bc}	50.59^{b}c	59.77^{a}	0.48	<0.001	<0.001
a* value	6.40^{c}	8.33^{ab}	6.47^{c}	6.22^{c}	8.30^{ab}	7.07^{bc}	9.11^{a}	6.87^{bc}	6.06^{c}	0.16	<0.001	<0.001
b* value	6.00^{b}	4.20^{c}	4.98b^{c}	4.54^{c}	4.39^{c}	4.20^{c}	5.28^{bc}	7.51^{a}	7.52^{a}	0.15	<0.001	<0.001
Sensory color$^{1)}$	3.08^{ab}	3.28^{a}	2.83^{ab}	3.30^{a}	3.40^{a}	2.67^{ab}	2.88^{ab}	2.43^{b}	2.79^{ab}	0.17	0.002	0.061
Marbling$^{2)}$	3.50^{a}	3.33^{a}	3.43^{a}	2.70^{ab}	3.08^{a}	2.15^{b}	3.35^{a}	3.03^{a}	3.33^{a}	0.07	<0.001	0.058
Pork quality classes (%)												
PSE pork	20.0	0.0	0.0	0.0	0.0	13.3	0.0	6.7	40.0	-	-	-
RSE pork	6.7	0.0	0.0	20.0	0.0	13.3	0.0	0.0	33.3	-	-	-
RFN pork	20.0	86.7	66.7	46.7	93.3	40.0	53.3	60.0	0.0	-	-	-
PFN pork	53.3	13.3	33.3	33.3	6.7	33.4	46.7	33.3	26.7	-	-	-
DFD pork	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-

$^{a-e}$Means in the same row with different superscripts differ ($p < 0.05$)

LT, low air temperature (lower than 10℃); NT, normal temperature(10℃ to 24℃); HT, high temperature(higher than 24℃)

LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg); HD, high density (higher than 0.37

m^2/100 kg)

1) Color score ranged from 1 (pale color) to 5 (dark color)

2) Marbling score ranged from 1 (practically devoid) to 5 (abundant)

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

Table 9. Effects of stocking density on pig behaviors, skin temperature, and respiratory frequency during pre-slaughter pig transport

	LD	ND	HD	SE	P-value
Basic behavior (min/hour)					
Standing	50.98	50.98	50.00	0.25	0.182
Sitting	5.70[b]	5.24[b]	9.00[a]	0.27	<0.001
Lying	3.33[a]	3.78[a]	1.01[b]	0.22	<0.001
Singularity behavior (count/hour)					
Aggression	5.90[a]	5.07[b]	6.40[a]	0.21	0.035
Overlap	6.13[b]	5.91[b]	7.67[a]	0.24	0.004
Respiratory frequency (count/min)					
Respiratory frequency	63.12[a]	59.89[b]	63.56[a]	0.39	<0.001
Skin temperature (℃)					
Before transport	37.43	37.41	37.33	0.02	0.115
After transport	39.50	39.60	39.60	0.07	0.379
Skin temperature change	2.07[b]	2.10[b]	2.27[a]	0.03	0.021

[a-b]Means in the same row with different superscripts differ ($p < 0.05$)

LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg); HD, high density (higher than 0.37 m^2/100 kg)

Table 10. Effects of air temperature on pig behaviors, skin temperature, and respiratory frequency during pre-slaughter pig transport

	LT	NT	HT	SE	P-value
Basic behavior (min/hour)					
Standing	52.42^{a}	50.07^{b}	49.46^{b}	0.25	<0.001
Sitting	6.42^{ab}	7.56^{a}	5.95^{b}	0.27	0.043
Lying	1.16^{c}	2.37^{b}	4.60^{a}	0.22	<0.001
Singularity behavior (count/hour)					
Aggression	6.13^{a}	4.88^{b}	6.37^{a}	0.21	0.008
Overlap	7.60^{a}	5.80^{b}	6.31^{ab}	0.24	0.006
Respiratory frequency (count/min)					
Respiratory frequency	60.32^{b}	61.03^{b}	65.21^{a}	0.39	<0.001
Skin temperature (°C)					
Before transport	37.40	37.42	37.36	0.02	0.506
After transport	39.26^{c}	39.57^{b}	39.87^{a}	0.03	<0.001
Skin temperature change	1.86^{c}	2.15^{b}	2.42^{a}	0.03	<0.001

$^{a\text{-}b}$Means in the same row with different superscripts differ ($p < 0.05$)

LT, low air temperature (lower than 10℃); NT, normal temperature (10℃ to 24℃); HT, high temperature (higher than 24℃)

Table 11. Effects of interaction between stocking density and air temperature on pig behaviors, skin temperature, and respiratory frequency during pre-slaughter pig transport

	LT			NT			HT			SE	P-value	
	LD	ND	HD	LD	ND	HD	LD	ND	HD		Treatments	Interaction
Basic behavior (min/hour)												
Standing	53.33^{a}	53.27^{a}	50.67^{ab}	50.27^{b}	50.80^{ab}	49.13^{b}	49.33^{b}	48.87^{b}	50.17^{b}	0.25	<0.001	0.025
Sitting	5.70^{cd}	4.73^{d}	8.83^{ab}	6.65^{bcd}	5.75^{cd}	10.29^{a}	4.73^{d}	5.25^{cd}	7.85^{abc}	0.27	<0.001	0.620
Lying	0.97^{c}	2.00^{bc}	0.51^{c}	3.09^{b}	3.45^{b}	0.57^{c}	5.93^{a}	5.88^{a}	1.97^{bc}	0.22	<0.001	0.001
Singularity behavior (count/hour)												
Aggression	7.70^{ab}	5.60^{bc}	5.07^{c}	5.10^{c}	4.13^{c}	5.40^{bc}	4.90^{c}	5.47^{bc}	8.73^{a}	0.21	<0.001	<0.001
Overlap	7.20^{ab}	6.40^{ab}	9.20^{a}	5.07^{b}	5.00^{b}	7.33^{ab}	6.13^{b}	6.33^{ab}	6.47^{ab}	0.24	0.001	0.291
Respiratory frequency (count/min)												
Respiratory frequency	60.70^{cd}	59.40^{cd}	60.87^{cd}	63.03^{bc}	58.13^{d}	61.92^{bcd}	65.63^{ab}	62.13^{bcd}	67.87^{a}	0.39	<0.001	0.098
Skin temperature (°C)												
Before transport	37.42	37.41	37.36	37.49	37.42	37.34	37.39	37.40	37.28	0.02	0.596	0.933
After transport	39.26^{de}	39.30^{cde}	39.23^{e}	39.59^{bcd}	39.57^{cde}	39.55^{cde}	39.65^{bc}	39.95^{ab}	40.02^{a}	0.03	<0.001	0.054
Skin temperature change	1.84^{d}	1.89^{cd}	1.87^{cd}	2.10^{bc}	2.15^{b}	2.21^{b}	2.26^{b}	2.27^{b}	2.74^{a}	0.03	<0.001	0.001

$^{a-e}$Means in the same row with different superscripts differ ($p < 0.05$)

LT, low air temperature (lower than 10°C); NT, normal temperature (10°C to 24°C); HT, high temperature (higher than 24°C)

LD, low density (lower than 0.43 m^2/100 kg); ND, normal density (0.37 m^2/100 kg to 0.43 m^2/100 kg); HD, high density (higher than 0.37 m^2/100 kg)

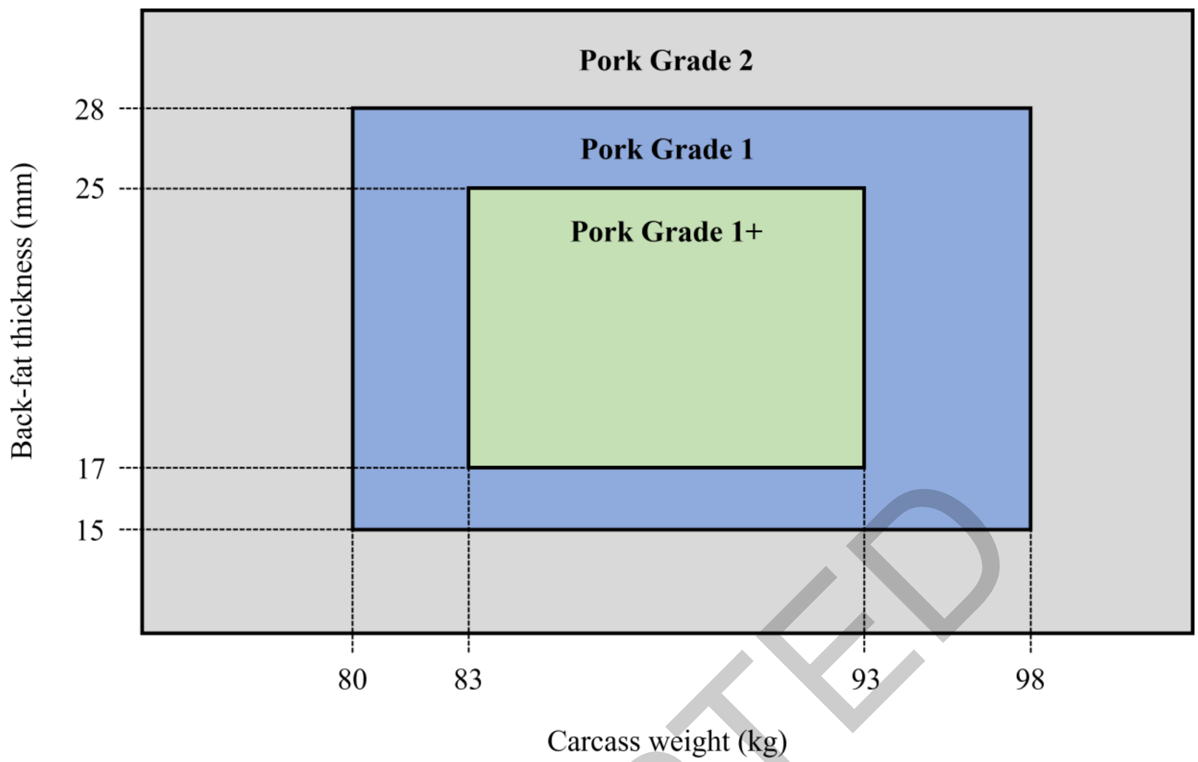

Figure 1. Korean carcass grading system according to carcass weight and back-fat thickness [19]

Figure 2. Korean marbling grading diagram according to instramuscular fat [26]