

JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	The Impact of Overnight Lairage on Meat Quality and Storage Stability of Pork Loin
Running Title	The Impact of Overnight Lairage on Pork Quality and Stability
Author	Minwoo Choi ¹ , Dongheon Lee ¹ , Hyun Jung Lee ¹ , Ki-Chang Nam ² , Sung-Sil Moon ³ , Jong Hyun Jung ^{4*} , Cheorun Jo ^{1, 5, 6*}
Affiliation	1. Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea 2. Department of Animal Science and Technology, Suncheon National University, Suncheon 57922, Korea 3. Sunjin Technology & Research Institute, Icheon 17332, Republic of Korea 4. Jung P&C Institute, Inc., Yongin 16950, Republic of Korea 5. Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea 6. Department of Animal Product Technology, Universitas Padjadjaran, West Java 45363, Indonesia
ORCID	Minwoo Choi (https://orcid.org/0000-0002-5459-3724) Dongheon Lee (https://orcid.org/0000-0002-6214-9295) Hyun Jung Lee (https://orcid.org/0000-0002-6891-8008) Ki-Chang Nam (https://orcid.org/0000-0002-2432-3045) Sung-Sil Moon (https://orcid.org/0000-0003-2734-8931) Jong Hyun Jung (https://orcid.org/0000-0003-3667-7710) Cheorun Jo (https://orcid.org/0000-0003-2109-3798)
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.	Not applicable.
Acknowledgements	This work was supported by the Korea Evaluation Institute of Industrial Technology (KEIT) through the Alchemist project funded by the Ministry of Trade, Industry and Energy (MOTIE; 20012411).
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Jong Hyun Jung, Ki-Chang Nam, Sung-Sil Moon, Cheorun Jo. Data curation: Hyun Jung Lee, Formal analysis: Minwoo Choi, Dongheon Lee Methodology: Minwoo Choi, Dongheon Lee, Ki-Chang Nam Software: Minwoo Choi. Validation: Hyun Jung Lee, Jong Hyun Jung, Sung-Sil Moon Investigation: Minwoo Choi, Dongheon Lee. Writing-original draft: Minwoo Choi. Writing-review & editing: Minwoo Choi, Dongheon Lee, Hyun Jung Lee, Sung-Sil Moon, Ki-Chang Nam, Jong Hyun Jung, Cheorun Jo.
Ethics approval and consent to participate	The experiment was approved by the institutional animal care and use committee (IACUC) at Suncheon National University, Suncheon, Republic of Korea (SCNU IACUC-2020-12).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jong Hyun Jung ¹ , Cheorun Jo ² ,
Email address – this is where your proofs will be sent	1. jhjih7@snu.ac.kr 2. cheorun@snu.ac.kr
Secondary Email address	
Address	1. 1504-1505, 120, Heungdeokjungang-ro, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea 2. 200-5218, 1 Gwanak-ro, Gwanak-gu, Seoul 08826
Cell phone number	1. +82-10-8704-2853 2. +82-10-3727-6923
Office phone number	1. +82-31-704-8113 2. +82-2-880-4804
Fax number	1. +82-31-705-0296 2. +82-2-873-2271

ACCEPTED

Abstract

Lairage, a part of the animal welfare practices, has been known to mitigate pre-slaughter stress in animals. However, research investigating the relationship between lairage and pork meat quality remains scarce. In this study, we conducted a comparative analysis of the physicochemical quality and storage stability of pork from pigs subjected to immediate slaughter (CON) and those provided with a 24 h lairage before slaughter (LRG) over a 7-day storage period. The loins from 20 castrated pigs in each group, respectively, were collected at 1, 3, 5, and 7 days and used for analysis of meat quality and storage stability, including pH, meat color, moisture, water holding capacity, drip loss, cooking loss, shear force, fatty acid composition, lipid oxidation, antioxidant activity, and electrical resistance. Overall, there were no significant differences in physicochemical meat quality parameters between CON and LRG groups. Similarly, no differences were observed in the storage stability of pork including 2-thiobarbituric acid reactive substances, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and electrical resistance. However, the proportion of unsaturated fatty acids was significantly higher in LRG compared to CON. In conclusion, 24 h lairage for castrated pigs had limited impact on meat quality and storage stability but led to an increase in the unsaturated fatty acid proportion.

Keywords: Animal Welfare, Lairage, Pork, Meat quality, Storage stability

Introduction

Over the past 20 years, consumer demand for animal welfare has witnessed a substantial increase. Ethical consciousness among consumers has reached a point where they refuse purchasing products failing to meet their animal welfare standards (Broom, 1986). Furnols (2019) suggested that meat quality now encompasses socio-ethical values, including consideration like production system, animal welfare, and environmental impacts, with animal welfare emerging as an important factor influencing consumer purchasing decisions. Furthermore, it is widely known that preslaughter stress can exert adverse effects on meat quality parameters, such as ultimate pH and drip loss (Ferguson, 2001). Consequently, effective control of preslaughter stress is imperative to ensure optimal meat quality (Hambrecht, 2005). Previous studies have shown that the implementation of animal welfare practices helps alleviate stress in animal as evidenced by indicators such as blood cortisol levels or heart rates (Brundige et al., 1998; Correa et al., 2010; Hemsworth et al., 2002). As a result, the importance of animal welfare has been consistently emphasized.

Pigs are commonly known to be sensitive to stress (Gregory, 1981). Therefore, various forms of stress can significantly affect the quality of pork (Dokmanovic, 2014). Many previous studies have consistently affirmed that stress experienced during the preslaughter period exerts adverse effects on pork quality. These effects include lower pH levels, reduced water holding capacity (WHC) and moisture content, along with higher drip loss, electrical conductivity, and L^* value (Van der Wal et al., 1999; Hambrecht et al., 2004; Rivas et al., 2020). For these reasons, it becomes evident that minimizing stress in domestic animals is significantly important. Accordingly, many studies have focused on developing strategies to alleviate the preslaughter stress (Hambrecht et al., 2005; Driessen et al., 2020; Delsart, 2020).

Lairage, an important preslaughter handling process that provides a rest period for animals

before they are slaughtered to reduce stress and fatigue that they may have experienced during transportation and therefore this process ultimately prevents quality deterioration (Hemsworth et al., 2002). Extensive research has been conducted on the effects of lairage on animal stress and meat quality, with a specific focus on the duration of lairage. Liste et al. (2011) suggested that lamb meat with 12 h lairage showed lower levels of cortisol, lactate, and glucose compared to meat without lairage. Additionally, it exhibited higher WHC and lower b^* value. Diaz et al. (2014) showed similar results that suckling lamb with 24 h lairage had lower b^* value and higher WHC than lamb without lairage. Moreover, they also indicated that, after five days of storage, the differences of meat color and WHC between the groups became reduced. Another previous study (Warriss, 1982) proposed that pre-slaughter fasting could be useful for increasing ultimate pH and lowering the possibility of pale, soft, and exudative (PSE) pork. However, the existing literature usually did not consider the above aspects as the main arguments of the paper; instead, it predominantly focused on stress indicators like cortisol (Velarde et al., 2015). Therefore, there is scarce information regarding the relationship between meat quality and lairage, especially in pork. In addition, as mentioned before, while stress is influence on the composition of the pork, limited research explores the relationship between lairage and storage stability of pork.

In this regard, we hypothesized that lairage may enhance pork quality during storage and improve storage stability. Therefore, we aimed to examine the effects of overnight lairage on quality characteristics and storage stability-related properties during 7 days of storage by comparing samples of pigs without lairage.

Materials and Methods

Animals and experimental design

The experimental design of the present study is described in Fig. 1. A total of 40 castrated pigs were raised in an animal-friendly farm, Cheil breeding stock farm (Incheon, Korea) until their live weight reached 30 kg. A stocking density of the farm was over 0.3 m² per animal. Then, they were transported to Yuunwoo animal welfare-friendly farm (Hwasung, Korea) and raised in four separate pens ensuring a stocking density of over 1 m² per animal until they reached the target weight of 110 kg. Each pen accommodated 10 pigs, with 5 of them undergoing an overnight lairage for 24 h without access to food, but with free access to water before being slaughtered (LRG). The pigs assigned to LRG were transported one day prior slaughter, while the other 5 pigs were slaughtered immediately upon arrival at the abattoir (CON). As a result, a total of twenty pigs were allocated to each treatment group. The abattoir (Dodram, Anseong, Korea) was approximately 90 km far from the farm, which took approximately 2 h for pig transportation. During transportation, the temperature was maintained in the enclosed truck at around 15-18°C to minimize stress. The detailed information of carcasses is shown in Table 1. Both groups were slaughtered on the same day, and the loin cuts were collected, vacuum-packed, and transported to the laboratory in a cooler with ice both top and bottom. The loins were stored for 1, 3, 5, and 7 days to investigate the effect of storage on pork loin from CON and LRG. The experiment was approved by the institutional animal care and use committee (IACUC) at Suncheon National University, Suncheon, Republic of Korea (SCNU IACUC-2020-12).

pH

A meat sample (2 g) was mixed with 18 mL of distilled water and homogenized using a homogenizer (Polytron PT10-35 GT, Kinematica AG, Luzern, Switzerland) at 11,000 rpm for 60 s. The homogenates were filtered using a Whatman No. 4 filter paper (Whatman PLC., Kent,

UK) and the pH of each filtrate was measured using a pH meter (SevenExcellence™, Mettler Toledo International Inc., Schwerzenbach, Switzerland). Before measurement, the pH meter was calibrated using standard buffers with pH values of 4.01, 7.00, and 9.21.

Meat color

Meat color was measured using a colorimeter (CM-5, Konica Minolta Sensing Inc., Osaka, Japan). Before measurement, the sample was exposed to air for 30 min to bloom, and the instrument was calibrated using a standard white plate. The illuminant was D65 and the standard observer was set to 10°, and the meat color was measured using a plate with a diameter of 30 mm. The results were expressed as Commission Internationale d'Eclairage (CIE) L^* , a^* , and b^* , which represent lightness, redness and yellowness, respectively. Each sample was measured three times and its average value was used as one replicate.

Moisture content

The moisture content was measured according to the AOAC method (1999) with some modification. The weight of a pre-dried aluminum dish was measured and three grams of meat sample was placed on the dish. The sample was then dried in a dry oven at 104°C until it reached a constant weight, and then transferred to the desiccator. The weight of the dried sample with the dish was measured. The moisture content was calculated as a percentage using the following equation:

$$\text{Moisture (\%)} = \frac{(P_0 + M_0) - P_1}{M_0} \times 100$$

where M_0 represents the weight of the sample. P_0 and P_1 represent the weight of the dish before

drying and the weight of the dish with sample after drying, respectively.

Water holding capacity (WHC)

Five grams of the sample were centrifuged at 1,000 ×g for 10 min at 5°C (Combi-514R, Hanil, Incheon, Korea). The weight of the meat sample was then measured, and the WHC was calculated using the following equation:

$$\text{WHC(\%)} = \frac{M \times S_1}{M \times S_0} \times 100$$

where M represents the value for moisture content, S_0 represents the initial sample weight, and S_1 represents the weight of the sample after centrifugation.

Drip loss and cooking loss

Drip loss was calculated by measuring the ratio of weight loss before and after storage (Kwon et al., 2022). Cooking loss was calculated by measuring the weight difference before and after heating a sample (30 mm × 50 mm × 10 mm; width × length × height) until its core temperature reached 75°C using water bath. The calculation formula is as follows:

$$\text{Cooking loss (\%)} = \frac{C_0 - C_1}{C_0} \times 100$$

where C_0 is the initial weight of the sample and C_1 is the weight of the sample after cooking.

Warner-Bratzler shear force

The sample was cut into a size of 40 mm × 50 mm × 10 mm (width × length × height), then

cooked until the core temperature of the sample reached 75°C. After that, the sample was re-cut into a size of 10 mm × 20 mm × 10 mm (width × length × height) to make the shape accurate and consistent (Kim et al., 2022). The Warner-Bratzler blade was attached to a texture analyzer (TA-XT2, Stable Micro System, Surrey, UK) and the muscle fiber direction of the sample was placed perpendicularly to the blade to measure the shear force. The instrumental condition was set to pre-test speed 2.0 mm/s, test speed 2.0 mm/s, and post-test speed 5.0 mm/s. The shear force was expressed as kgf.

Fatty acid composition

Fatty acid composition of the meat sample was measured according to O'fallon et al. (2007) and Uyen et al. (2023) with some modification. The sample (1 g) was mixed with 0.7 mL of 10 N KOH and 6.3 mL of methanol and placed in a constant-temperature water bath at 55°C. The mixture was vigorously shaken every 30 min for 1 h and 30 min. After cooling in ice water for 1-2 min, 0.58 mL of 24 N H₂SO₄ was added to the samples and the mixture was shaken again every 30 min for 1 h and 30 min while being heated in the constant-temperature water bath at 55°C. After cooling in ice water, 3 mL of hexane was added, and the mixture was centrifuged at 3,000 ×g for 5 min using a Combi-514R centrifuge (Hanil Co., Ltd., Daejeon, Korea) to separate the fatty acid methyl esters. The upper layer was transferred to a vial using a Pasteur pipette, injected into the gas chromatograph equipped with the HP-88 column (60 m × 0.2 mm × 250 µm) and a flame ionization detector (Agilent 7890 series, Wilmington, USA). The injector was set to a split ratio of 25:1 with a temperature of 250°C, and the temperature of the detector was set to 250°C. A high-purity mixture of air, H₂, and He was used as a carrier gas with a flow rate of 400 mL/min for air and 40 mL/min for H₂.

2-Thiobarbituric acid reactive substances (TBARS)

The sample (5 g) was mixed with 15 mL of distilled water in a 50 mL test tube and homogenized. Then, 1 mL of the homogenate was transferred into a disposable test tube, and 50 µL of 7.2% butylated hydroxytoluene in ethanol (w/v) and 2 mL of 20 mM thiobarbituric acid (TBA) in 15% trichloroacetic acid (TCA) solution (w/v) were added into the tube. The mixture was thoroughly vortexed and incubated at 95°C in a water bath for 15 min, and cooled for 10 min. The mixture was mixed again, and centrifuged at 3,000 rpm at 5°C for 15 min (Combi-514R, Hanil Co., Ltd.). The absorbance of the supernatant was measured at 531 nm. Distilled water (1 mL) and TBA/TCA solution (2 mL) were mixed together to be used as a blank. The TBARS value was expressed as mg malondialdehyde (MDA) per kg of meat sample.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The meat sample (2 g) was mixed with 18 mL of distilled water and homogenized at 11,000 rpm for 1 min using a homogenizer (Polytron PT10-35 GT, Kinematica AG). The homogenate was then centrifuged at 3,000 × g for 10 min (Combi-514R, Hanil Co., Ltd.). After centrifugation, 0.4 mL of the supernatant was mixed with 1.6 mL of distilled water and 2 mL of 0.2 mM DPPH in methanol solution. The mixture was incubated at room temperature for 60 min in the dark, and the absorbance of the sample was measured at 517 nm. Ascorbic acid was used as a reference and distilled water absorbance was also measured for control. Lastly, the DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Electrical resistance

Each sample's electrical resistance was measured using an RCL electric bridge (630A automatic RCL meter with an adaptor PM 9542A, Philips, Germany). The measurement was performed by inserting a probe (YL-69, China), which is a soil moisture sensor, into the center of the sample to obtain the impedance (Z , Ω) value. The measurement was repeated three times to obtain the mean value.

Statistical analysis

The results were analyzed via one- and two-way analysis of variance using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Tukey's multiple test was used to determine the significant differences between sample groups at $p < 0.05$.

Results and Discussion

Comparative analysis of the physicochemical characteristics of CON and LRG groups

We conducted a comparative analysis of the differences in the physicochemical quality of the CON and LRG groups over a 7-day storage period, including pH, meat color, moisture, WHC, drip loss, cooking loss, and shear force (Table 2).

pH

There were no significant differences in pH between the CON and LRG groups during five days of storage, but on day 7, the LRG group showed significantly higher pH compared to CON ($p < 0.05$) (Table 2). The overall pH values of pork were within an average pH range of pork loin

(from 5.5 to 5.8) which was reported by Warner et al. (1997), indicating that lairage did not directly affect the incidence of pale, soft, and exudative (PSE) or DFD meat. It was hard to find any research comparing samples that underwent no lairage before slaughter and those with a 24 h lairage (long period lairage). However, the previous study (Dokmanovic, 2014) compared short and long period lairage indicated that there was no significant difference observed in the ultimate pH of pork between short (8 min to 2.7 h) and extended period lairage (14 h to 21.5 h). Furthermore, Fernandez et al. (1992) and Smiecinska et al. (2011) reached similar conclusions, finding no significant difference in pH between groups subjected to short lairage and those with an extended lairage period. This suggests that the pork production system can accommodate an overnight lairage period as a pre-slaughter method, followed by the findings outlined in the earlier paper by Miranda-de la Lama et al. (2009).

The pork samples were stored for days 1-7 to simulate retail condition, and during storage, the meat pH within each group did not differ significantly during seven days of storage. Importantly, meat pH is strongly correlated with other physicochemical quality attributes such as meat color and WHC (Klont, 2005). Therefore, the similar pH value between both groups might lead to the similar meat color and WHC observed in our study. Meanwhile, as Liste (2011) stated, lairage could reduce the levels of cortisol, lactate, and glucose. These are stress metabolites, and if their blood levels are high, it leads to excessive postmortem muscle metabolism and lowers the pH level (Čobanović et al., 2020). However, since there was no difference in this study, further research is needed from a metabolite perspective.

Meat color

There were no significant differences in any of the L^* , a^* , and b^* values between CON and

LRG groups at all storage periods ($p > 0.05$) except for L^* value on day 5, where CON showed higher value than LRG ($p < 0.05$) (Table 2). Within the CON group, the L^* value decreased on day 3 but then increased on day 5, while the a^* value increased on day 3 and decreased on day 7. The b^* value showed no significant changes throughout the storage period in CON. Conversely, in the LRG group, the L^* value decreased on day 3, then increased on day 5. Regarding the a^* value of LRG, it increased on day 3 but gradually decreased on days 5 and 7. Similar to CON, the b^* value in LRG showed no significant changes during the storage period. Furthermore, a two-way ANOVA (Table S1) revealed that the presence of lairage only had a significant effect on L^* value, while storage time significantly affected all meat color traits in both in CON and LRG. These findings align with a previous study (Zhen et al., 2013), which compared the meat qualities of pigs subjected to four different lairage times (0 h, 3 h, 8 h, and 24 h). Their study found that the initial L^* value was higher in 0 h lairage group than in 24 h lairage group, which was in accordance with our results on day 5. However, the initial a^* value was lower in 0 h group. Lastly, the b^* value did not show significant difference between 0 h and 24 h groups, which corresponds to our result. In another study (Nanni Costa et al., 2002), the L^* value and a^* value of pork loin between 2 h and overnight lairage groups were not significantly different.

Moisture and WHC

The moisture content was not significantly different between CON and LRG groups throughout the whole storage period except for day 5, where CON had a significantly higher value than LRG ($p < 0.01$) (Table 2). In CON group, the highest moisture content was observed on day 3, with no significant differences noted on the other days. Similarly, in LRG, the highest value was also observed on day 3, with no significant differences on the other days.

Similarly, there were no significant differences in WHC were observed throughout the entire storage period. In CON group, the WHC was the highest at the initial storage day, with no significant differences between days 3, 5, and 7. Conversely, in LRG, the highest value of WHC was observed on day 1 but gradually decreased, showing significant differences on days 5 and 7. The distribution of water within the muscle tissue includes both intra-myofibrillar and extra-myofibrillar spaces of the cells, causing changes in the intracellular structure that directly impact the WHC. Moreover, as postmortem rigor progresses, the space within the myofibrils decreases gradually, leading to the extrusion of water and resulting in a higher drip loss.

Drip loss and cooking loss

Both drip loss and cooking loss did not show significant differences throughout the entire storage period between CON and LRG (Table 2). In CON group, drip loss significantly increased from day 3 to day 5 ($p < 0.05$), while cooking loss showed a significant increase over the storage period, reaching its highest value on day 5, followed by a significant decrease on day 7. Similar trends in the changes of drip loss and cooking loss were observed in LRG. Drip loss is closely related with pH and WHC (Klont, 2005; Huff-Loneragan, 2005); higher pH and WHC resulted in less drip loss. Hence, the similar pH and WHC values observed in both CON and LRG groups likely contributed to the absence of significant difference in drip loss and cooking loss.

Warner-Bratzler shear force

Except for day 7 where CON showed a lower value than LRG ($p < 0.05$), there were no significant difference in the shear force value between CON and LRG ($p > 0.05$) (Table 2). In CON group, the shear force decreased gradually from 5.70 on the initial day of storage to 3.02 on

day 7 ($p < 0.05$). Similarly, in LRG, the shear force also showed a gradual decrease from 6.01 on day 1 to 3.32 on day 7 during storage ($p < 0.05$). Both groups exhibited a decrease in shear force values with increasing storage days, which can be attributed to the degradation of Z-disks in myofibrillar structures and the weakening of actin-myosin interactions caused by enzymes which are present in the meat (Olson et al., 1977; Bhat et al., 2018).

Overall, the effect of lairage on the physicochemical meat quality attributes was not significant in this study.

Differences in fatty acids composition on lairage duration

Overall, there were no significant differences in the fatty acid composition between CON and LRG (Table 3). According to the previous study (Piedrafita et al., 2001), differences in fatty acid composition were observed depending on the stress genotype of pigs. In the study, the stress-positive pigs had the lower percentages of the C14 and C16 fatty acids (C14:0, C16:0, and C16:1), but higher proportion of C18:0 acid than in the stress-negative pigs. In present study, although the percentage of an individual fatty acid did not show any significant difference, the sum of unsaturated fatty acids was significantly higher in LRG compared to CON. As a result, LRG showed significantly lower atherogenic index (AI) and thrombogenicity index (TI) compared to CON. It has been known that unsaturated fatty acids have various effects on cancer, diabetes, cardiovascular disease and even on cognitive function (Lunn, 2006). Furthermore, lower values of AI and TI indicate better nutritional lipid quality as these indexes indicate the reduced probability of the incidence of atherosclerotic and thrombus formation, respectively (Ke et al., 2022). Therefore, it could be interpreted that overnight lairage might have some positive impact on human health to some extent.

Comparative analysis of the storage stability of pork from lairage

Lastly, to examine the differences in storage stability between CON and LRG during storage, we measured parameters including TBARS as an index of the degree of lipid oxidation, DPPH radical scavenging activity, and electrical resistance, which is known to be effective in freshness prediction (Zhang, 2022). For this analysis, it was only measured at day 1 and day 7, representing the initial and final day of whole storage for comparing changes in storage stability.

TBARS values were significantly higher on day 7 compared to day 1 in both the CON and LRG groups, with no significant difference between the two groups (Table 4). On the other hand, DPPH radical scavenging activity showed no significant differences between days 1 and 7 within each group, and similarly, no significant difference observed between the two groups. These findings suggest that a 24 h lairage did not significantly influence on the oxidation of meat.

According to Zhang (2022), the tissue structure or cellular morphology of meat during storage can impact electrical parameters, and therefore electrical resistance holds potential predicting meat freshness like total volatile basic nitrogen (TVB-N) value. In CON group, the electrical resistance decreased significantly from day 1 to 7 (Table 4). Similarly, in LRG, there was a significant decrease in electrical resistance from day 1 to day 7, with no significant difference between the two groups. Therefore, considering the results of TBARS, DPPH, and electrical resistance, it appears that overnight lairage did not show a significant effect on storage stability of pork.

Previous studies have suggested that overnight lairage may lead to more frequent instances of fighting among pigs and increased occurrence of skin blemishes (Brown et al., 1999). Additionally, it was proposed that the optimal lairage time for pigs could be between 1 and 3 h

(Warriss et al., 1998). Although significant differences were not observed in most quality indicators between CON and LRG groups in the present study, the results could contribute to the establishment of more strategic conditions for animal welfare in pig production. In addition, it may be advisable to consider exploring the effect of a variety of lairage periods from short to long periods in future studies.

Acknowledgments

This work was supported by the Korea Evaluation Institute of Industrial Technology (KEIT) through the Alchemist project funded by the Ministry of Trade, Industry and Energy (MOTIE; 20012411).

- 327 Bhat ZF, Morton JD, Mason SL, Bekhit AEDA. Role of calpain system in meat tenderness: A
 328 review. Food Sci Hum Wellness. 2018;7(3):196-204.
 329 <https://doi.org/10.1016/j.fshw.2018.08.002>
- 330 Broom DM, Indicators of poor welfare. Brit. Vet. J. 1986;142:524-526.
 331 [https://doi.org/10.1016/0007-1935\(86\)90109-0](https://doi.org/10.1016/0007-1935(86)90109-0).
- 332 Brown SN, Knowles TG, Edwards JE, Warriss PD. Behavioural and physiological responses of
 333 pigs to being transported for up to 24 hours followed by six hours recovery in lairage. Vet.
 334 1999;145:421–426. <https://doi.org/10.1136/vr.145.15.421>.
- 335 Brundige L, Oleas T, Doumit M, Zanella AJ. Loading techniques and their effect on behavioral
 336 and physiological responses of market weight pigs. J Anim Sci. 1998;76:99 (Abstr.)
- 337 Čobanović N, Stanković SD, Dimitrijević M, Suvajdžić B, Grković N, Vasilev D, Karabasil N.
 338 Identifying physiological stress biomarkers for prediction of pork quality variation. Animals,
 339 2020;10(4):614. <https://doi.org/10.3390/ani10040614>
- 340 Correa JA, Torrey S, Devillers N, Laforest JP, Gonyou HW, Faucitano L. Effects of different
 341 moving devices at loading on stress response and meat quality in pigs. J Anim Sci.
 342 2010;88:4086–4093. <https://doi.org/10.2527/jas.2010-2833>
- 343 Delsart M, Pol F, Dufour B, Rose N, Fablet C. Pig farming in alternative systems: strengths and
 344 challenges in terms of animal welfare, biosecurity, animal health and pork safety.
 345 Agriculture. 2020;10(7):261. <https://doi.org/10.3390/agriculture10070261>
- 346 Díaz MT, Vieira C, Pérez C, Lauzurica S, De Chávarri EG, Sánchez M, De la Fuente J. Effect of
 347 lairage time (0 h, 3 h, 6 h or 12 h) on glycogen content and meat quality parameters in
 348 suckling lambs. Meat Sci. 2014;96(2):653-660.
 349 <https://doi.org/10.1016/j.meatsci.2013.10.013>
- 350 Dokmanović M, Velarde A, Tomović V, Glamočlija N, Marković R, Janjić J, Baltić MŽ. The
 351 effects of lairage time and handling procedure prior to slaughter on stress and meat quality
 352 parameters in pigs. Meat Sci. 2014;98(2):220-226.
 353 <https://doi.org/10.1016/j.meatsci.2014.06.003>
- 354 Driessen B, Freson L, Buyse J. Fasting finisher pigs before slaughter influences pork safety, pork
 355 quality and animal welfare. Animals. 2020;10(12):2206.
 356 <https://doi.org/10.3390/ani10122206>

357 Ferguson DM, Bruce HL, Thompson JM, Egan AF, Perry D, Shorthose WR. Factors affecting
358 beef palatability—farmgate to chilled carcass. *Aust J Exp Agric.* 2001;41(7):879-891.
359 <https://doi.org/10.1071/EA00022>

360 Fernandez X, Mågård M, Tornberg E. The variation in pig muscle glycolytic potential during
361 lairage-An in-vivo study. *Meat Sci.* 1992;32(1):81-91. [https://doi.org/10.1016/0309-](https://doi.org/10.1016/0309-1740(92)90018-Y)
362 [1740\(92\)90018-Y](https://doi.org/10.1016/0309-1740(92)90018-Y).

363 Font-i-Furnols M, Skrlep M, Aluwé M. Attitudes and beliefs of consumers towards pig welfare
364 and pork quality. *IOP Conf. Ser.: Earth Environ Sci.* 2019;333(1):012002.
365 <https://doi.org/10.1088/1755-1315/333/1/012002>

366 Gregory NG, Lister D. Autonomic responsiveness in stress-sensitive and stress-resistant pigs. *J*
367 *Vet Pharmacol Ther.* 1981;4(1):67-75. <https://doi.org/10.1111/j.1365-2885.1981.tb00712.x>

368 Hambrecht, E, Eissen JJ, Nooijen RIJ, Ducro BJ, Smits CHM, Den Hartog LA, Verstegen MWA.
369 Preslaughter stress and muscle energy largely determine pork quality at two commercial
370 processing plants. *J Anim Sci.* 2004;82(5):1401-1409.
371 <https://doi.org/10.2527/2004.8251401x>

372 Hambrecht E, Eissen JJ, Newman DJ, Smits CHM, Den Hartog LA, Verstegen MWA. Negative
373 effects of stress immediately before slaughter on pork quality are aggravated by suboptimal
374 transport and lairage conditions. *J Anim Sci.* 2005;83(2):440-448.
375 <https://doi.org/10.2527/2005.832440x>

376 Hambrecht E, Eissen JJ, Newman DJ, Smits CHM, Verstegen MWA, Den Hartog LA.
377 Preslaughter handling effects on pork quality and glycolytic potential in two muscles
378 differing in fiber type composition. *J Anim Sci.* 2005;83(4):900-907.
379 <https://doi.org/10.2527/2005.834900x>

380 Hemsworth PH, Barnett JL, Hofmeyr C, Coleman GJ, Dowling S, Boyce J. The effects of fear of
381 humans and pre-slaughter handling on the meat quality of pigs. *Aust J Agric Res.*
382 2002;53(4):493-501. <https://doi.org/10.1071/AR01098>

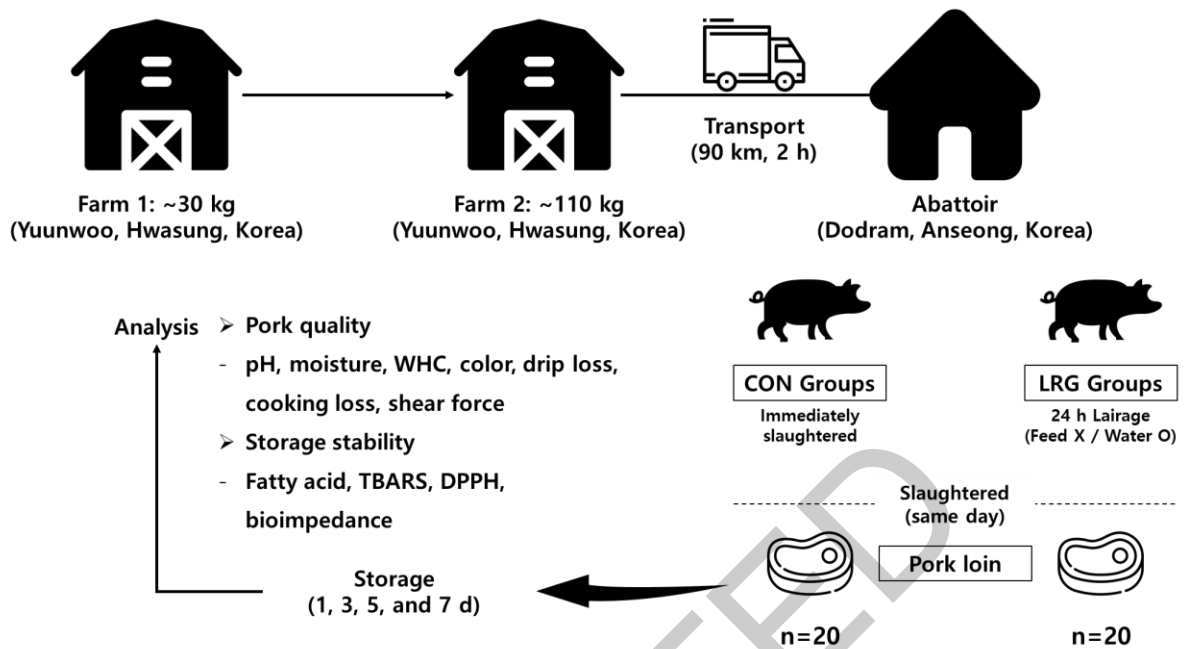
383 Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat: The role of
384 postmortem biochemical and structural changes. *Meat Sci.* 2005;71(1):194-204.
385 <https://doi.org/10.1016/j.meatsci.2005.04.022>

386 Ke Z, Bai Y, Bai Y, Chu Y, Gu S, Xiang X, Ding Y, Zhou X. Cold plasma treated air improves the
387 characteristic flavor of Dry-cured black carp through facilitating lipid oxidation. *Food*
388 *Chem.* 2022;377, 131932. <https://doi.org/10.1016/j.foodchem.2021.131932>

- 389 Kim SS, Lee YE, Kim CH, Min JS, Yim DG, Jo C. Determining the optimal cooking time for
390 cooking loss, shear force, and off-odor reduction of pork large intestines. *Food Sci. Anim*
391 *Resour.* 2022;42(2):332. <https://doi.org/10.5851/kosfa.2022.e6>
- 392 Klont R. Influence of ultimate pH on meat quality and consumer purchasing decisions. PIC
393 [www.thepigsite.com/articles/6/production-and-mgmt/1506/influence-of-ultimate-ph-on-](http://www.thepigsite.com/articles/6/production-and-mgmt/1506/influence-of-ultimate-ph-on-meat-quality-and-consumer-purchasing-decisions)
394 [meat-quality-and-consumer-purchasing-decisions](http://www.thepigsite.com/articles/6/production-and-mgmt/1506/influence-of-ultimate-ph-on-meat-quality-and-consumer-purchasing-decisions) [Accessed: August 2023]. 2005.
- 395 Kwon JA, Yim DG, Kim HJ, Ismail A, Kim SS, Lee HJ, Jo C. Effect of temperature abuse on
396 quality and metabolites of frozen/thawed beef loins. *Food Sci Anim Resour.* 2022;42(2):341.
397 <https://doi.org/10.5851/kosfa.2022.e9>
- 398 Liste G, Miranda-De La Lama GC, Campo MM, Villarroel M, Muela E, María GA. Effect of
399 lairage on lamb welfare and meat quality. *Anim Prod Sci.* 2011;51(10):952-958.
400 <https://doi.org/10.1071/AN10274>
- 401 Lunn J, Theobald HE. The health effects of dietary unsaturated fatty acids. *Nutr Bull.*
402 2006;31(3):178-224. <https://doi.org/10.1111/j.1467-3010.2006.00571.x>
- 403 Miranda-De La Lama GC, Villarroel M, Olleta JL, Alierta S, Sañudo C, Maria GA. Effect of the
404 pre-slaughter logistic chain on meat quality of lambs. *Meat Sci.* 2009;83(4):604-609.
405 <https://doi.org/10.1016/j.meatsci.2009.07.009>
- 406 Nanni Costa L, Fiego DL, Dall'Olio S, Davoli R, Russo V. Combined effects of pre-slaughter
407 treatments and lairage time on carcass and meat quality in pigs of different halothane
408 genotype. *Meat Sci.* 2002;61(1):41-47. [https://doi.org/10.1016/S0309-1740\(01\)00160-7](https://doi.org/10.1016/S0309-1740(01)00160-7)
- 409 O'Fallon JV, Busboom JR, Nelson ML, Gaskins CT. A direct method for fatty acid methyl ester
410 synthesis: Application to wet meat tissues, oils, and feedstuffs. *J Anim Sci.*
411 2007;85(6):1511-1521. <https://doi.org/10.2527/jas.2006-491>
- 412 Olson DG, Parrish Jr FC, Dayton WR, Goll DE. Effect of postmortem storage and calcium
413 activated factor on the myofibrillar proteins of bovine skeletal muscle. *J Food Sci.*
414 1977;42(1):117-124. <https://doi.org/10.1111/j.1365-2621.1977.tb01233.x>
- 415 Piedrafita J, Christian LL, Lonergan SM. Fatty acid profiles in three stress genotypes of swine
416 and relationships with performance, carcass and meat quality traits. *Meat Sci.*
417 2001;57(1):71-77. [https://doi.org/10.1016/S0309-1740\(00\)00078-4](https://doi.org/10.1016/S0309-1740(00)00078-4)
- 418 Gonzalez-Rivas PA, Chauhan SS, Ha M, Fegan N, Dunshea FR, Warner RD. Effects of heat
419 stress on animal physiology, metabolism, and meat quality: A review. *Meat Sci.*

- 2020;162(1):108025. <https://doi.org/10.1016/j.meatsci.2019.108025>
- Smiecinska K, Denaburski J, Sobotka W. Slaughter value, meat quality, creatine kinase activity and cortisol levels in the blood serum of growing finishing pigs slaughtered immediately after transport and after a rest period. *Pol. J. Vet. Sci.* 2011;14(1):47-54. <https://doi.org/10.2478/v10181-011-0007-x>
- Uyen NT, Van Cuong D, Thuy PD, Son LH, Ngan NT, Quang NH, Tuan ND, Hwang IH. A Comparative Study on the Adipogenic and Myogenic Capacity of Muscle Satellite Cells, and Meat Quality Characteristics between Hanwoo and Vietnamese Yellow Steers. *Food Sci Anim Resour.* 2023;43(4):563. <https://doi.org/10.5851/kosfa.2023.e19>
- Van der Wal PG, Engel B, Reimert HM. The effect of stress, applied immediately before stunning, on pork quality. *Meat Sci.* 1999;53(2):101-106. [https://doi.org/10.1016/S0309-1740\(99\)00039-X](https://doi.org/10.1016/S0309-1740(99)00039-X)
- Velarde A, Fàbrega E, Blanco-Penedo I, Dalmau A. Animal welfare towards sustainability in pork meat production. *Meat Sci.* 2015;109:13-17. <https://doi.org/10.1016/j.meatsci.2015.05.010>
- Warriss PD, Brown SN, Edwards JE, Knowles TG. Effect of lairage time on levels of stress and meat quality in pigs. *Anim. Sci.* 1998;66:255–26. <https://doi.org/10.1017/S1357729800009036>
- Warner RD, Kauffman RG, Greaser ML. Muscle protein changes post mortem in relation to pork quality traits. *Meat Sci.* 1997;45(3):339-352. [https://doi.org/10.1016/S0309-1740\(96\)00116-7](https://doi.org/10.1016/S0309-1740(96)00116-7)
- Warriss PD. Loss of carcass weight, liver weight and liver glycogen, and the effects on muscle glycogen and ultimate pH in pigs fasted preslaughter. *J. Sci. Food Agric.* 1982;33:840–846. <https://doi.org/10.1002/jsfa.2740330905>
- Zhang Z, Li X, Tian J, Chen J, Gao G. A review: Application and research progress of bioimpedance in meat quality inspection. *J Food Process Eng.* 2022;45(11):e14153. <https://doi.org/10.1111/jfpe.14153>
- Zhen S, Liu Y, Li X, Ge K, Chen H, Li C, Ren F. Effects of lairage time on welfare indicators, energy metabolism and meat quality of pigs in Beijing. *Meat Sci.* 2013;93(2):287-291. <https://doi.org/10.1016/j.meatsci.2012.09.008>

451 **Figure legends**



452
453 Figure 1. A schematic diagram of the experiment design. On day 1, 20 pigs designated for the lairage
454 (LRG) group were brought from the farm and held in a lairage area for 24 h. On day 2, 20 pigs designated
455 for the control (CON) group were brought from the same farm to the abattoir directly. Both groups were
456 slaughtered on the same day, and carcasses from each group were randomly selected and loin cuts from
457 the carcasses were collected. Each loin cut was divided into four blocks and stored for 1, 3, 5, and 7 days
458 and used for pork quality and storage stability analyses. WHC, water holding capacity; TBARS, 2-
459 thiobarbituric acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

460

461

462 Table 1. The carcass characteristics and fat content of the pigs with no (CON) or 24 h lairage (LRG)

Treatment	Backfat depth (mm)	Carcass weight (kg)	Fat content (%)
CON	22.20 ^b	88.05	3.38
LRG	23.25 ^a	89.50	3.36
SEM	0.297	0.611	0.233

463 ^{a, b} Different letters within the same column indicate significant difference ($p < 0.05$).

464 SEM. standard error of the mean (n = 40).

ACCEPTED

465 Table 2. Changes in the physicochemical traits of pork loin from the pigs with no (CON) or 24 h lairage
466 (LRG) during seven days of storage

Trait	Treatment	Storage period (d)				SEM ¹⁾
		1	3	5	7	
pH	CON	5.70	5.68	5.70	5.68 ^b	0.018
	LRG	5.74	5.74	5.76	5.74 ^a	0.028
	SEM ²⁾	0.026	0.025	0.023	0.020	
CIE <i>L</i> [*]	CON	55.72 ^X	54.61 ^Y	56.11 ^{aX}	56.98 ^X	0.389
	LRG	54.85 ^X	54.12 ^Y	55.45 ^{bX}	56.44 ^X	0.409
	SEM ²⁾	0.368	0.361	0.390	0.428	
CIE <i>a</i> [*]	CON	15.89 ^{YZ}	16.76 ^X	16.39 ^{XY}	15.19 ^Z	0.194
	LRG	15.80 ^{YZ}	16.91 ^X	16.18 ^Y	15.29 ^Z	0.159
	SEM ²⁾	0.181	0.158	0.126	0.206	
CIE <i>b</i> [*]	CON	5.40	5.37	5.83	5.93	0.168
	LRG	5.56	5.21	5.61	5.68	0.153
	SEM ²⁾	0.136	0.104	0.167	0.217	
Moisture (%)	CON	74.11 ^Y	74.82 ^X	74.00 ^{aY}	73.73 ^Y	0.174
	LRG	73.61 ^Y	74.53 ^X	73.26 ^{bY}	73.51 ^Y	0.196
	SEM ²⁾	0.191	0.188	0.158	0.201	
WHC (%)	CON	69.19 ^X	64.28 ^Y	63.53 ^Y	63.18 ^Y	0.975
	LRG	69.32 ^X	65.06 ^{XY}	62.57 ^Y	61.35 ^Y	1.164
	SEM ²⁾	0.717	1.104	1.115	1.279	
Drip loss (%)	CON	-	3.81 ^Y	6.42 ^X	7.47 ^X	0.369 ³⁾

	LRG	-	3.36 ^Y	5.99 ^X	7.05 ^X	0.381 ³⁾
	SEM ²⁾	-	0.289	0.397	0.426	
Cooking loss (%)	CON	20.74 ^Z	25.33 ^Y	27.93 ^X	24.58 ^Y	0.479
	LRG	21.06 ^Z	24.99 ^Y	28.07 ^X	24.46 ^Y	0.466
	SEM ²⁾	0.260	0.502	0.610	0.448	
Shear force (kgf)	CON	5.70 ^X	4.19 ^Y	3.54 ^{YZ}	3.02 ^{bZ}	0.178
	LRG	6.01 ^X	4.38 ^Y	3.66 ^Z	3.32 ^{aZ}	0.174
	SEM ²⁾	0.259	0.177	0.130	0.091	

^{a, b} Different letters within the same column indicate significant difference ($p < 0.05$).

^{x-z} Different letters within the same row indicate significant difference ($p < 0.05$).

SEM, standard error of the mean ¹⁾ (n = 80), ²⁾ (n = 40), and ³⁾ (n = 60).

WHC; water holding capacity.

471 Table 3. Fatty acid composition (%) of pork loins with no (CON) or 24 h lairage (LRG)

	CON	LRG	SEM
C _{10:0}	0.13	0.12	0.003
C _{12:0}	0.11	0.11	0.002
C _{14:0}	1.55	1.47	0.330
C _{16:0}	24.91	24.32	0.228
C _{16:1}	3.31	3.33	0.092
C _{18:0}	12.07	11.63	0.218
C _{18:1}	42.99	43.94	0.604
C _{18:2}	9.69	9.61	0.395
C _{18:3}	0.70	0.70	0.020
C _{20:2}	0.32	0.31	0.009
C _{20:3}	0.25	0.25	0.016
C _{20:4}	1.84	1.92	0.164
C _{20:5}	0.04	0.05	0.003
C _{22:6}	0.04	0.04	0.003
C _{24:1}	0.32	0.35	0.026
Total	98.26	98.14	0.193
SFA	38.76	37.65	0.394
UFA	59.49 ^Y	60.49 ^X	0.339
MUFA	46.62	47.62	0.626
PUFA	12.88	12.87	0.550

UFA/SFA	1.54 ^Y	1.61 ^X	0.025
n-6/n-3	11.50	11.45	0.481
n-6	11.84	11.84	0.542
n-3	1.03	1.03	0.021
AI	0.53 ^X	0.50 ^Y	0.008
TI	1.19 ^X	1.14 ^Y	0.018
P/S	0.33	0.34	0.017

^{X, Y} Different letters within the same row indicate significant difference ($p < 0.05$).

SEM, standard error of the mean ($n = 30$).

SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA; polyunsaturated fatty acid; AI, atherogenic index; TI, thrombogenicity index; P/S, ratio of PUFA to SFA.

476 Table 4. Changes in the TBARS, antioxidant activity, and electrical resistance of pork loin from the pigs
477 with no (CON) or 24 h lairage (LRG) during seven days of storage

Trait	Treatment	Storage period (d)		SEM
		1	7	
TBARS (mg MDA/kg)	CON	0.17 ^Y	0.21 ^X	0.004
	LRG	0.18 ^Y	0.21 ^X	0.003
	SEM	0.004	0.003	
DPPH radical scavenging activity (%)	CON	48.88	48.55	0.788
	LRG	49.44	49.09	1.117
	SEM	1.021	0.909	
Electrical resistance {Auto (Ω)}	CON	205.60 ^X	117.82 ^Y	5.740
	LRG	203.00 ^X	118.45 ^Y	4.465
	SEM	7.143	1.367	

478 ^{X, Y} Different letters within the same row indicate significant difference ($p < 0.05$).

479 SEM, standard error of the mean (n = 40).

480 TBARS, 2-thiobarbituric reactive substances; MDA, malondialdehyde; DPPH, 2,2-diphenyl-1-
481 picrylhydrazyl.