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Running Title (within 10 words)	Lairage Temperature and Duration Effects on Pork Quality
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Abstract

This study investigated the interactive effects of lairage duration and environmental temperature on physiological stress responses, behavioral patterns, and meat quality characteristics in finishing pigs. A total of 1,809 crossbred pigs (Duroc × [Yorkshire × Landrace]) weighing 115-120 kg were subjected to two lairage durations (0-4 h vs. 4-8 h) under three environmental temperatures: low temperature (LT, <10°C), normal temperature (NT, 10-24°C), and high temperature (HT, >24°C). Results demonstrated that environmental temperature significantly affected cortisol concentrations (p<0.001), with LT conditions producing elevated stress responses compared to NT and HT treatments. Lairage duration influenced lactate accumulation patterns, indicating differential energy metabolism dynamics across temporal treatments. Interactive effects between temperature and duration were observed for multiple parameters including pH values (p<0.001), glucose concentrations (p=0.036). Water-holding capacity showed significant temperature-dependent variations (p<0.001), while meat color yellowness was significantly affected by thermal conditions (p<0.001), and lightness was significantly affected by lairage duration (p<0.01). Behavioral observations revealed that HT exposed pigs exhibited increased lying behavior and reduced standing time compared to other groups, reflecting thermoregulatory adaptations. Aggressive behaviors and overlap activities were significantly affected by environmental temperature (p<0.001), with HT pigs showing markedly reduced frequencies compared to LT and NT pigs. The incidence of quality defects, particularly PSE characteristics, numerically increased under HT conditions. These findings demonstrate that optimal lairage management requires integrated consideration of both temporal and thermal factors rather than addressing these variables independently. The results emphasize the need for flexible, context-specific protocols that account for environmental conditions and their interactions to maintain animal welfare and product quality under varying commercial conditions.

Keywords: pig, lairage, temperature, stress, meat quality, animal welfare

Introduction

In the modern swine industry, producing high-quality pork is essential for enhancing consumer satisfaction and boosting farm profitability. Despite advancements in genetics and rearing management, stress during the pre-slaughter phase continues to be a significant factor affecting meat quality and carcass defects [1, 2]. Specifically, the transport of pigs from farms to slaughterhouses and the subsequent lairage period cause rapid physical and psychological changes, disrupting their physiological homeostasis [3, 4]. Lairage is a critical process that allows pigs to recover from the energy depletion experienced during transport and regain stability, helping maintain muscle glycogen levels. Adequate rest is vital for producing quality meat, as it lowers cortisol levels and regulates post-mortem glycolysis [5, 6]. However, inadequate lairage conditions or extended durations can have negative consequences. Mixing unfamiliar pigs incites aggressive behaviors as they establish social hierarchies, which can lead to skin lesions that degrade carcass quality and increase lactate accumulation due to heightened physical activity [7, 8]. These acute stress responses can cause a rapid decline in early post-mortem pH, raising the likelihood of producing PSE (Pale, Soft, Exudative) meat or resulting in DFD (Dark, Firm, Dry) meat due to glycogen depletion from prolonged stress [9, 10]. Pigs are particularly sensitive to temperature fluctuations because they lack functional sweat glands. Research indicates that during summer, when heat stress is common, meat quality often deteriorates due to hyperventilation and increased metabolism for heat dissipation, while winter conditions raise energy demands for thermoregulation [11, 12]. Most studies have focused on the effects of season or lairage duration separately; however, there is a lack of research on the complex interactions between temperature and lairage duration and their effects on behavioral responses and meat quality [13, 14]. Therefore, this study aims to evaluate the effects of different lairage durations under varying seasonal temperature conditions on behavioral changes and the severity of skin damage in finishing pigs. Additionally, physiological responses assessed through blood stress indicators, as well as carcass grading outcomes and the physicochemical properties of the meat, were examined to provide practical guidance for lairage management.

Materials and Methods

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-2185-23-02).

Animals, treatments and pre-slaughter conditions

This study was conducted in the Republic of Korea, over a period of one year All pigs were reared on the same commercial farm and transported to the same commercial slaughterhouse. A total of 1,809 mixed-sex crossbred fattening pigs (Duroc × [Yorkshire × Landrace], LYD) with live weights ranging from 115 to 120 kg were used. Pigs were transported to the slaughterhouse over a total of 15 trips throughout the one-year experimental period, with each trip covering a distance of approximately 40 km. On the day of the slaughter, pigs were moved early in the morning from the farm to the slaughterhouse. Upon arrival at the slaughterhouse, pigs were randomly allocated to lairage pens according to their assigned lairage duration treatment: short lairage (S, 0–4 h) or long lairage (L, 4–8 h). Pigs that were transported together as a group were maintained in the same lairage pen without mixing with unfamiliar conspecifics, in order to minimize additional social stress. Although slaughter times varied slightly among groups, all pigs within each trip were slaughtered on the same day. Every pig was slaughtered in accordance with the regulations set forth by the Livestock Sanitation Management Act. The experiment was conducted under three ambient temperature conditions reflecting seasonal variation: high temperature (HT, >24°C), low temperature (LT, <10°C), and normal temperature (NT, 10–24°C). Indoor lairage temperature was equivalent to the prevailing outdoor ambient temperature at the time of each trial. Each lairage pen had a floor area of 38.44 m^2, accommodating pigs at a stocking density of 1.2 pigs/m^2. All management procedures, feeding regimens, and animal handling protocols remained consistent across all seasonal periods to minimize potential confounding effects.

Carcass quality measurements

The pig carcasses grade system followed the Korean Pig Carcass Grade System [15]. An electronic scale was used for 45 min postmortem to measure the weight of the hot carcass weight, which was showed in integer kg units.

Backfat thickness was determined by using a left half carcass. Additionally, the length between the 11th and 12th thoracic vertebrae, the first lumbar vertebra, and the last thoracic vertebrae was measured by using a ruler, in order to assess the thickness of the backfat thickness. The formula (backfat thickness [mm] per hot carcass weight [kg]) was used for calculating the hot carcass weight and backfat thickness.

Pork composition and quality

According to the standard recommendation by the AOAC International [16], the moisture, protein, and fat content (%) was analyzed. To measure the pH, 50 mL of distilled water was added to 5 g of the left carcass loin. After homogenizing all of the samples for 30 s with a homogenizer, the pH of each sample was measured using an Orion StarTM A211 pH Benchtop Meter (Thermo Fisher Scientific Inc., USA), which was calibrated at pH 4, 7, and 10. Left carcass loin was analyzed with a Spectro Colorimeter standardized on a white plate (L*, 89.39; a*, 0.13; b*, -0.51) to determine the meat color. During this period, a white-fluorescent lamp (D65) served as the light source. Color values were featured as L* (lightness), a* (redness), b* (yellowness). Drip loss (DL) was determined by using the filter paper wetness test. The weight loss that occurred when suspending a standardized muscle sample (40 - 50 g, or about 30 mm × 60 mm × 25 mm) in an airtight container for 48 h at 4 °C was quantified as DL, and the weight disparities were taken into account. Cooking loss (CL) was determined. For determination of CL, a meat sample was divided into 1 × 1 × 1 cm from each animal. Samples were weighed and then kept overnight at 4 °C. The samples were continuously cooled to ambient temperature once the sample′s external temperature reached 85 °C, and then they were weighed to determine the CL. The weight of the initial sample divided by the weight of the heated sample to calculate the CL value, which showed as a percentage. Following the Koćwin-Podsiadła et al. [17] methodology, pork quality classes were measured by using light reflectance (L*), DL fluctuations, and pH values evaluated 24 h postmortem.

Behavior observation

Real-time pig behavior monitoring was conducted using cameras placed on the lairage ceiling to record continuous footage throughout the entire lairage period. Behavioral observations were performed using instantaneous scan sampling by a single trained observer who was blinded to the treatment allocation of each group. The position of pigs was divided into basic behavior (standing, sitting, and lying) and singularity behavior (aggression, overlap). Description of pig behavior followed Song et al. [18] methodology. Only recordings with a minimum of 10 pigs in each group that could be clearly seen were examined for the measurement because the compartment group was not always entirely seen by the camera.

Blood profile

Blood samples were collected immediately upon jugular vein severance during the exsanguination process at slaughter, from 10 pigs per treatment group. Although blood collection at exsanguination may introduce acute stress responses associated with the slaughter procedure itself, this method is widely adopted in pre-slaughter stress research as it represents the most feasible and ethically accepted approach for obtaining blood in a commercial slaughter setting and has been validated in previous studies as a reliable indicator of cumulative pre-slaughter stress [4]. Serum samples were centrifuged at 3,000 × g for 20 min at 4°C and stored at −20°C until analysis. Serum cortisol concentration was determined using a radioimmunoassay Coat-A-Count kit (Siemens, USA) according to the manufacturer's instructions. Glucose concentration was measured using an automatic Kone-lab analyzer, and lactate concentration was determined using a GM7 Analox analyzer.

Statistical analysis

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Temperature condition (HT, NT, LT), lairage duration (S, L), and their interaction were specified as fixed effects, and the 15 transportation events as a random effect. pH, WHC, DL, CL, instrumental meat color, and blood profile — were analyzed by two-way ANOVA using the GLM procedure. Treatment means were compared by Tukey's multiple range test, and variability was reported as the pooled standard error. Pork quality classes and carcass grades were analyzed using generalized linear mixed models with a binomial distribution and logit link function, retaining the same fixed and random-effect structure. Main effects were tested by chi-square tests, and the temperature × lairage interaction by a likelihood-ratio test. For the DFD class, in which observations were confined to a single temperature–lairage cell, Firth's penalized maximum likelihood estimation and Fisher's exact test were applied to address quasi-complete separation. Frequencies are presented as percentages with the pooled SE. Statistical significance was declared at $p < 0.05$, and a tendency at $0.05 \leq p < 0.10$.

Results

Carcass Traits and Composition

Hot carcass weight was significantly higher ($p < 0.001$) in HT pigs than in LT pigs, while lairage duration had no significant effect ($p = 0.157$). Backfat thickness did not differ significantly across temperature or lairage duration treatments. Regarding proximate composition, moisture content was significantly higher ($p = 0.027$) in LT pigs than in HT pigs, while crude protein and crude fat were not significantly affected by temperature. Crude fat tended to be higher ($p = 0.080$) in pigs subjected to long lairage compared to short lairage.

Meat Quality Characteristics

Muscle pH was significantly higher ($p = 0.003$) in LT pigs than in HT pigs, with NT recording intermediate values, while lairage duration had no significant effect ($p = 0.532$). WHC was significantly lower ($p < 0.001$) in LT pigs than in NT and HT pigs, with no significant difference between NT and HT groups. DL and CL did not differ significantly across treatments. Lightness (L*) was significantly higher ($p = 0.003$) in pigs subjected to long lairage than in those with short lairage, whereas temperature had no significant effect ($p = 0.176$). Yellowness (b*) was significantly lower ($p < 0.001$) in NT pigs than in LT and HT pigs, while lairage duration had no significant effect. Regarding pork quality class distribution, PSE incidence was significantly affected by lairage duration ($p = 0.008$), with short lairage pigs (12.59%) showing higher incidence than long lairage pigs (3.70%). PFN proportion was also significantly influenced by lairage duration ($p = 0.014$), with long lairage pigs (25.19%) showing higher values than short lairage pigs (13.33%). DFD occurrence was significantly affected by temperature ($p = 0.048$), being observed exclusively in LT pigs (3.33%). No significant temperature × lairage interactions were detected for any quality class.

Behavioral Characteristics

Standing time was significantly lower ($p < 0.001$) in HT pigs than in LT pigs. Conversely, lying time was significantly different ($p < 0.001$) among all three temperature groups, with HT pigs showing the highest values, followed by NT and LT pigs. Sitting time was significantly higher ($p = 0.040$) in LT pigs than in NT pigs, with HT pigs showing intermediate values. Lairage duration had no significant effect on basic postural behaviors.
Aggression frequency was significantly lower ($p < 0.001$) in HT pigs than in LT and NT pigs, while LT and NT pigs did not differ significantly. Lairage duration also significantly influenced aggression frequency ($p < 0.001$). Overlap

frequency was significantly different ($p < 0.001$) among all three temperature groups, with LT pigs showing the highest values, followed by NT and then HT pigs, with no significant effect of lairage duration ($p = 0.990$).

Blood Profiles

Cortisol concentration was significantly higher ($p < 0.001$) in LT pigs than in NT and HT pigs, while NT and HT pigs did not differ significantly. Pigs subjected to short lairage showed significantly higher ($p = 0.005$) cortisol concentrations than those with long lairage.

Lactic acid concentration was significantly higher ($p < 0.001$) in LT pigs than in NT and HT pigs, while NT and HT pigs did not differ significantly. Lairage duration had no significant effect on lactic acid ($p = 0.076$).

Glucose concentration was significantly higher ($p = 0.003$) in LT and NT pigs than in HT pigs. Pigs subjected to short lairage showed significantly higher ($p = 0.048$) glucose concentrations than those with long lairage.

Discussion

This study provides evidence that cold stress during lairage poses a greater physiological challenge than previously recognized, and that temperature and lairage duration interact selectively on postmortem muscle metabolism.

Hot carcass weight was significantly higher in HT pigs than in LT pigs, with NT intermediate. Although carcass weight is primarily determined during the growing–finishing phase [19], the lower weight in LT pigs may partly reflect increased metabolic expenditure from cold-induced thermogenesis, as shivering and peripheral vasoconstriction elevate energy consumption [11]. However, seasonal differences in on-farm feed intake could confound this interpretation, and the absolute difference is modest. The absence of significant effects on backfat thickness and carcass grade distribution confirms that acute preslaughter thermal conditions have limited influence on compositional traits [20].

Among meat quality parameters, the most notable finding was the paradox between pH and WHC. LT pigs had the highest ultimate pH yet the lowest WHC, whereas NT and HT pigs with lower pH values showed significantly higher WHC. Conventionally, higher ultimate pH is associated with greater WHC due to increased net charge of myofibrillar proteins [21]. However, under LT conditions, accelerated carcass cooling may have induced cold shortening sarcomere contraction at low muscle temperatures that reduces water retention regardless of ultimate pH [22, 23]. The higher pH in LT pigs is attributable to glycogen depletion from sustained shivering, which limited substrate for postmortem glycolysis [10, 24]. The significant T × L interaction on pH ($p < 0.001$) and L* ($p = 0.006$) indicates that postmortem glycolysis responds to the specific combination of thermal load and lairage duration, whereas WHC and DL showed no interaction, suggesting they are governed by thermal trajectory during rigor rather than ante-mortem stress combinations. Yellowness (b*) was lower in NT than in both LT and HT pigs, potentially reflecting elevated oxidative stress under both thermal extremes [25, 26].

Pork quality class analysis revealed that PSE and DFD are driven by distinct stressor types. PSE incidence was determined primarily by lairage duration, indicating that insufficient recovery time leaves residual catecholamine activity that accelerates early postmortem glycolysis [9]. Conversely, DFD occurred exclusively in LT pigs, consistent with chronic glycogen depletion from cold-stress thermogenesis [10, 27]. The higher PFN proportion in long-lairage pigs likely reflects progressive glycogen utilization during extended feed withdrawal. The absence of significant T × L interactions for any quality class suggests that PSE prevention requires adequate lairage time regardless of temperature, while DFD prevention requires thermal management independently of duration [14].

Behavioral responses showed a consistent temperature gradient: lying time increased progressively, indicating continuous thermoregulatory adjustment proportional to thermal load [11, 12]. The marked reduction of aggression and overlap under HT conditions is consistent with behavioral suppression driven by thermoregulatory priority rather than physical incapacity [28]. Notably, overlap frequency differentiated all three temperature groups while aggression did not differ between LT and NT, suggesting that mounting behavior is more sensitive to moderate thermal changes. The absence of lairage duration effects on postural behaviors indicates that thermoregulatory strategies persist throughout lairage, contrasting with the expectation that extended confinement increases restlessness [29].

Blood stress markers revealed that LT conditions produced the highest cortisol and lactic acid concentrations, significantly exceeding both NT and HT groups. This challenges the predominant focus on heat stress in preslaughter welfare research and indicates that cold-induced thermogenesis imposes a greater neuroendocrine burden than heat exposure [30, 31]. The moderate cortisol response under HT may reflect the effectiveness of behavioral thermoregulation in buffering physiological stress [4]. The significant lairage effect on cortisol confirms that adequate rest facilitates HPA-axis recovery. Glucose showed a significant T × L interaction, indicating that stress-induced gluconeogenesis and peripheral glucose consumption vary dynamically with both thermal load and lairage duration [32, 33].

In conclusion, this study demonstrates that cold stress during lairage poses a greater physiological challenge than heat stress, as evidenced by elevated cortisol, lactic acid, and exclusive DFD occurrence under LT conditions. Based on these findings, the following recommendations are proposed: under LT conditions (<10°C), extended lairage (4–8 h) with wind barriers or supplemental heating is recommended to mitigate glycogen depletion; under HT conditions (>24°C), adequate ventilation and water access should be prioritized rather than extending lairage duration; under NT conditions (10–24°C), standard 4–8 h protocols remain adequate. Regardless of temperature, a minimum lairage exceeding 4 hours is recommended to reduce PSE incidence. These temperature-specific protocols provide a practical framework for season-adjusted lairage management.

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Tables and Figures

Table 1. Effects of lairage time on carcass traits at different temperatures

Items	T			L		SEM	p-value		
	LT	NT	HT	0–4 h	4–8 h		T	L	T × L
Carcass composition									
Hot carcass weight, kg	86.54^{b}	88.39^{ab}	87.90^{a}	87.80	87.42	0.404	<0.001	0.157	0.355
Backfat thickness, mm	22.89	22.43	22.51	22.64	22.58	0.361	0.239	0.792	0.513
Carcass grade score, %[1]									
Grade 1+	37.68	37.69	40	39.44	37.65	–	0.657	0.456	0.686
Grade 1	33.93	35.26	33.39	32.54	35.55	–	0.799	0.198	0.572
Grade 2	28.39	27.05	26.61	28.02	26.80	–	0.784	0.579	0.347

T, temperature; L, lairage time; LT, low temperature (<10°C); NT, normal temperature (10–24°C); HT, high temperature (>24°C); 0–4 h, short lairage (0–4 h); 4–8 h, long lairage (4–8 h); T × L, interaction between temperature and lairage duration.

[a,b] Means in the same row with different superscripts differ significantly ($p < 0.05$) by Tukey's multiple range test.

[1] Carcass grade score distribution was compared by chi-square test (section-level p-values apply to joint distribution of all grades). Grade 1+: 83–93 kg carcass weight with 17–25 mm backfat; Grade 1: 80–98 kg with 15–28 mm backfat; Grade 2: all others.

Table 2. Effects of lairage time on pork compositions at different temperatures

Items	T			L		SEM	p-value		
	LT	NT	HT	0–4 h	4–8 h		T	L	T × L
Pork composition, %									
Moisture	74.15[a]	73.46[ab]	72.83[b]	73.39	73.57	0.484	0.027	0.639	0.102
Crude protein	22.09	21.88	21.24	22.03	21.45	0.440	0.138	0.108	0.418
Crude fat	2.19	2.32	2.27	2.17	2.34	0.161	0.533	0.080	0.807
Pork quality parameters									
pH	5.64[a]	5.55[ab]	5.46[b]	5.54	5.57	0.053	0.003	0.532	<0.001
WHC, %	62.63[b]	67.11[a]	67.21[a]	65.68	65.63	0.817	<0.001	0.940	0.516
DL, %	4.84	4.77	4.73	4.86	4.70	0.156	0.798	0.212	0.976
CL, %	24.71	24.53	23.77	24.48	24.19	0.415	0.058	0.405	0.380
L* value	53.24	51.84	52.74	51.68	53.53	0.762	0.176	0.003	0.006
b* value	6.99[a]	6.21[b]	6.98[a]	6.63	6.83	0.183	<0.001	0.172	0.871

T, temperature; L, lairage time; LT, low temperature (<10°C); NT, normal temperature (10–24°C); HT, high temperature (>24°C); 0–4 h, short lairage (0–4 h); 4–8 h, long lairage (4–8 h); T × L, interaction between temperature and lairage duration.

[a,b] Means in the same row with different superscripts differ.

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

Table 3. Effects of lairage time on pork quality at different temperatures

Items	T			L		SEM	p-value		
	LT	NT	HT	0–4 h	4–8 h		T	L	T × L
Pork quality classes, %									
PSE	8.89	7.78	7.78	12.59	3.70	–	0.952	0.008	0.783
RSE	7.78	3.33	6.67	6.67	5.19	–	0.422	0.606	0.617
RFN	58.89	71.11	66.67	67.41	63.70	–	0.218	0.522	0.251
PFN	21.11	17.78	18.89	13.33	25.19	–	0.846	0.014	0.205
DFD	3.33	0	0	0	2.22	–	0.048	0.082	-

T, temperature; L, lairage time; LT, low temperature (<10°C); NT, normal temperature (10–24°C); HT, high temperature (>24°C); 0–4 h, short lairage (0–4 h); 4–8 h, long lairage (4–8 h); T × L, interaction between temperature and lairage duration.

PSE, pale, soft, exudative; RSE, reddish–pink, soft, exudative; RFN, red, firm, nonexudative; PFN, pale, firm, nonexudative; DFD, dark, firm, dry.

Table 4. Effects of lairage time on behaviors at different temperatures.

Items	T			L		SEM	p-value		
	LT	NT	HT	0–4 h	4–8 h		T	L	T × L
Standing	36.91^{a}	35.34^{ab}	32.46^{b}	35.28	34.52	0.894	<0.001	0.294	0.462
Sitting	5.90^{a}	5.02^{b}	5.19^{ab}	5.27	5.47	0.366	0.040	0.574	0.950
Lying	17.19^{c}	19.64^{b}	22.35^{a}	19.43	20.03	0.95	<0.001	0.441	0.433
Aggression	3.69^{a}	2.87^{ab}	0.76^{c}	2.42	2.46	0.079	<0.001	<0.001	0.246
Overlap	1.92^{a}	1.57^{b}	0.56^{c}	1.32	1.38	0.076	<0.001	0.990	0.380

T, temperature; L, lairage time; LT, low temperature (<10°C); NT, normal temperature (10–24°C); HT, high temperature (>24°C); 0–4 h, short lairage (0–4 h); 4–8 h, long lairage (4–8 h); T × L, interaction between temperature and lairage duration.

$^{a-c}$ Means in the same row with different superscripts differ.

Table 5. Effects of lairage time blood profiles at different temperatures.

Items	T			L		SEM	p-value		
	LT	NT	HT	0–4 h	4–8 h		T	L	T × L
Cortisol, μg/dL	3.84^{a}	3.45^{bc}	3.61^{b}	3.75	3.52	0.077	<0.001	0.005	0.589
Lactic acid, mmol/L	4.83^{a}	3.96^{bc}	4.55^{b}	4.48	4.42	0.076	<0.001	0.076	0.078
Glucose, mg/dL	81.10	80.90	79.65	80.67	80.43	0.463	0.003	0.048	0.036

T, temperature; L, lairage time; LT, low temperature (<10°C); NT, normal temperature (10–24°C); HT, high temperature (>24°C); 0–4 h, short lairage (0–4 h); 4–8 h, long lairage (4–8 h); T × L, interaction between temperature and lairage duration.

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