

Effects of Pre-slaughter Transport Time and Lairage Time on Pig Stress and Meat Quality in Different Seasons

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3

4 **Abstract**

5 This study aimed to investigate the effects of different pre-slaughter transport durations
6 (1 h, 2.5 h, 4 h) and lairage times (12 h, 16 h) on stress levels and pork quality in pigs
7 under varying seasonal conditions. Within different seasons, pigs slaughtered in spring
8 showed the lowest overall stress levels and the best meat quality. Within the same
9 season, pigs transported for 2.5 h showed significantly lower levels of stress hormones
10 and metabolites (adrenocorticotrophic hormone, ACTH; cortisol, COR; glucose, GLU),
11 lower enzyme activity (creatine kinase, CK), lower drip loss, and lower cooking loss
12 compared to those transported for 1 h or 4 h. The pH of the meats for 2.5 h transportation
13 was significantly higher compared to 1 h and 4 h. Pigs lairaged for 12 h showed
14 significantly lower serum stress indicators and lower meat lightness (L*) compared to
15 those lairaged for 16 h. The differences in stress levels and meat quality between the
16 two lairage time groups were more pronounced during summer compared to other
17 seasons. In order to reduce pig stress and ensure pork quality, under short-distance
18 transport conditions, transport time of 2.5 h is better than 1 h or 4 h. During summer,
19 the lairage time in holding pens for 12 h is recommended, this is important to avoid
20 excessive physical exertion in pigs caused by prolonged fasting and high temperatures,
21 thereby safeguarding post-slaughter pork quality. The results of this study provide
22 significant information for the improvement of processing conditions for better meat
23 quality.

24
25 **Keywords:** pig slaughter, season, transport time, lairage time, stress, and pork quality.

1. Introduction

Stress response is a complex, multi-factor-induced, systematic and non-specific adaptive reaction of an animal organism to internal and external stimuli. The factors causing these responses are termed stressors (Holmes et al., 2021, Jackson *et al.*, 2021). The hypothalamic-pituitary-adrenal (HPA) axis serves as the primary endocrine axis mediating the stress response. Stress signals are transmitted to the paraventricular nucleus (PVN) of the hypothalamus. Subsequently, a subset of PVN neurons releases corticotropin-releasing hormone (CRH) into the pituitary portal system, stimulating the synthesis and release of adrenocorticotrophic hormone (ACTH) (Sautron et al., 2015). Upon reaching the adrenal cortex, ACTH promotes the synthesis of glucocorticoids, such as cortisol, which regulate the physiological responses to stressors (Oyola and Handa, 2017).

Studies have demonstrated that stress responses elevate glycolysis and gluconeogenesis rates, leading to increased blood glucose levels. Prolonged exposure to stress can consequently elevate blood lactate concentrations, resulting in decreased muscle pH. This cascade ultimately impairs water-holding capacity (WHC), causes pale coloration, and contributes to the development of pale, soft, exudative (PSE) meat. These changes significantly diminish the quality and market competitiveness of pork meat (Biggs et al., 2020, Pearce et al., 2013).

Current research on the impact of pre-slaughter management practices on stress responses and pork quality in pigs exists some inconsistencies. Therefore, this study aimed to systematically investigate stress levels experienced by pigs subjected to varying transport durations and lairage durations in different seasons. Specifically, we examined the effects of these stressors on postmortem pork quality. The findings provide a scientific basis and technical support for the swine slaughter industry to enhance pork quality and market competitiveness.

2. Materials and methods

2.1. Materials

Standard buffer solutions (pH 4.00 and 7.00) were obtained from Shanghai INESA Scientific Instrument Co., Ltd. (China). Enzyme membranes for biochemical analyzer: Glucose (GLU), Lactate (LAC), and Glutamate (Glu) detection membranes were supplied by Seamann Technology Co., Ltd. (China). Composite ion reagent kits, standard ion electrode solutions, and reference ion electrode solutions were provided by Seamann Technology Co., Ltd. (China). Commercial enzyme-linked immunosorbent assay (ELISA) kits for quantifying Creatine Kinase (CK), Creatinine (Cr), Alanine Aminotransferase (ALT), Adrenocorticotrophic Hormone (ACTH), Cortisol (COR), and Lactate Dehydrogenase (LDH) were purchased from Shanghai Jining Industrial Co., Ltd. (China). PHS-25 Benchtop pH Meter was from Shanghai INESA Scientific Instrument Co., Ltd., China. NV222ZH and PR223ZH Analytical Balances were from Ohaus Instrument Co., Ltd., USA. NR60CP Handheld Colorimeter was purchased from 3nh Technology Co., Ltd., China. ZBRTE-71B Constant-Temperature Water Bath was ordered from Changzhou Zhirui Instrument Manufacturing Co., Ltd., China. X1R High-Speed Refrigerated Centrifuge was from Thermo Fisher Scientific Inc., USA. M-900 Biochemical Analyzer was ordered from Seamann Technology Co., Ltd., China. W-960 Automated Microplate Washer (Wellwash Versa Microplate Washer) was from Huison Science and Technology Development Co., Ltd., China. A51119500C Full-Wavelength Microplate Reader was ordered from Thermo Fisher Scientific Inc., USA.

2.2. Animal

A total of 45 healthy Landrace pigs, weighing around 110 kg and qualified during pre-slaughter inspection, were used in this study. Slaughter procedures, including transport, lairage, and other slaughter-related procedures, were conducted under temperatures averaging 12°C in winter (January-February), 24°C in spring (April),

and 35°C in summer (July-August). Blood samples were collected to analyze stress-related biochemical indicators. Additionally, longissimus dorsi (LD) muscle samples were collected for pork quality assessment. The specific experimental groups for each season are listed in Table 1.

Table 1. Specific details of experimental groups in each season

Grouping Factor	Group Detail	Sample Size (n)
Transport Duration	Transport: 1 h (Fixed Lairage: 12 h)	3
	Transport: 2.5 h (Fixed Lairage: 12 h)	3
	Transport: 4 h (Fixed Lairage: 12 h)	3
Lairage Duration	Lairage: 12 h (Fixed Transport: 2.5 h)	3
	Lairage: 16 h (Fixed Transport: 2.5 h)	3

This study was carried out in accordance with Guidance on the Animals Act 1986 and associated guideline EU Directive 2010/63 for the protection of animals used for scientific purposes.

2.3. Methods

2.3.1. Sample Collection and Preparation

Concurrent with exsanguination, 40 mL of blood was collected from each pig into

50 mL centrifuge tubes. The blood was subsequently transferred using a 5 mL syringe into 5 mL coagulation-promoting tubes. Samples were allowed clotting at ambient temperature (approx. 25°C) for 3 hours. Serum was then isolated by centrifugation using a high-speed refrigerated centrifuge at 4°C, 1500 × g (relative centrifugal force), for 10 minutes. The isolated serum was transferred into 1.5 mL microcentrifuge tubes and stored at -20°C for subsequent analysis of biochemical indicators, enzyme activities, and hormone concentrations.

Immediately after postmortem, the longissimus dorsi (LD) muscle was collected between the 7th and 8th ribs on the left carcass half. Samples were transported to the laboratory immediately after collection for the measurement of pH, color (chroma), and water-holding capacity (WHC). Samples were then stored at 4°C. These measurements (pH, color, and WHC) were repeated after 24 hours.

2.3.2. Determination of Enzyme Activity, Hormone Levels, and Metabolite Concentrations

Serum levels of adrenocorticotrophic hormone (ACTH), cortisol (COR), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), and creatinine (Cr), as well as the enzyme activities of ALT, LDH, and CK, were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols. Measurements were performed using a full-wavelength microplate reader (Thermo Fisher A51119500C, USA).

2.3.3 Determination of Glucose, Lactate, Glutamate, and Ion Levels

Serum concentrations of glucose (GLU), lactate (LAC), glutamate (Glu), and composite ions were quantified using a biochemical analyzer (Seamann Technology M-900, China). Following preparation and 6-hour equilibration, the glucose buffer solution was transferred to the analyzer's designated buffer reservoir. Enzyme membranes for GLU, LAC, and Glu detection were installed into their respective silver disc enzyme electrodes and connected to the reaction chamber. Subsequently,

standard solutions for GLU, LAC, Glu, and composite ions were connected to their corresponding instrument inlets. The analyzer was then calibrated according to the manufacturer's protocol using the installed standards and buffer. Following successful calibration, thawed serum samples (in microcentrifuge tubes) were placed into the sample detection port. Each sample underwent triplicate measurements, and the mean value of these replicates was recorded as the final concentration for each analyte.

2.3.4 pH Measurement

Muscle pH was measured according to the method described by Lomiwes et al (Lomiwes et al., 2014). using a benchtop pH meter (Shanghai INESA Scientific Instrument Co., Ltd. PHS-25, China). The pH of the longissimus dorsi (LD) muscle was determined at 45 minutes postmortem (pH_{45 min}) and 24 hours postmortem (pH_{24 h}). Prior to each measurement session, the pH meter was calibrated with standard buffer solutions (pH 4.00 and 7.00). For each LD sample, pH measurements were taken at three distinct locations, and the mean value of these three replicates was calculated and recorded as the final pH value for subsequent data analysis.

2.3.5 Color Measurement

Muscle color was evaluated according to the method described by Bakhsh et al (Bakhsh et al., 2021). using an NR60CP handheld colorimeter (3nh Technology Co., Ltd., China). Instrument calibration was performed with a standard calibration tile prior to measurements. Color parameters of the longissimus dorsi (LD) muscle were assessed at 45 min postmortem and 24 h postmortem. Samples were removed from vacuum bags and surface moisture was immediately wiped dry with filter paper. Three consecutive measurements were taken at distinct locations on each sample surface. The mean values of lightness (L*), redness (a*), and yellowness (b*) from these triplicate measurements were calculated and recorded as result for statistical analysis.

2.3.6 Drip Loss Measurement

Drip loss percentage was determined following the methodology described by Żelechowska et al (Żelechowska et al., 2012). At 24 hours postmortem, longissimus dorsi (LD) muscle samples were sectioned into pieces measuring 5 cm × 3 cm × 2 cm and weighed to obtain the initial mass (m_1). Each sample was then placed in an individual plastic bag, suspended within a 4°C refrigerator, and carefully positioned to prevent contact with the bag walls. After 48 hours of suspension, samples were removed, surface moisture was gently wiped dry with absorbent paper, and the samples were reweighed to obtain the final mass (m_2). Drip loss percentage was calculated according to formula (1-1).

$$\text{Drip loss\%} = (m_1 - m_2) / m_1 \times 100\% \quad (1-1)$$

2.3.7 Cooking Loss Measurement

Longissimus dorsi (LD) muscle samples were sectioned into pieces measuring 5 cm × 3 cm × 2 cm and weighed to obtain the initial mass (m_3). Each sample was sealed within a heat-resistant plastic bag, submerged in a water bath maintained at 80°C (ZBRTE-71B Constant-Temperature Water Bath, Changzhou Zhirui Instrument Manufacturing Co., Ltd., China), and heated until the core temperature reached 75°C, monitored using a penetration thermometer. Heating continued at this core temperature for 20 minutes. Following cooking, samples were cooled to room temperature (approx. 25°C), removed from the bags, and surface exudate was gently wiped dry with absorbent paper. Samples were then reweighed to obtain the final cooked mass (m_4). Cooking loss percentage was calculated according to the following formula

$$\text{Cooking Loss\%} = (m_3 - m_4) / m_3 \times 100\% \quad (1-2)$$

2.3.8 Statistical Analysis

All experimental data were analyzed using SPSS Statistics 25.0 (IBM Corp., USA). Significant differences between groups were determined using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests for multi-group

comparisons, and independent samples t-tests for comparisons between two groups. Data are presented as the mean $\pm$ standard deviation (SD). Differences were considered statistically significant at $p < 0.05$ unless otherwise stated. Graphical representations of the data were generated using GraphPad Prism 8.0 (GraphPad Software, USA). In figures, different lowercase letters above bars or data points denote statistically significant differences between groups ($p < 0.05$), while shared letters indicate no significant difference ($p > 0.05$).

3. Result

3.1. Effects of Season and Transport Duration on Blood Physicochemical Indicators and Pork Quality

3.1.1. Effects of Season and Transport Duration on Serum Hormone Levels

As shown in Figure 1, during winter, pigs subjected to 1 h transport exhibited significantly higher serum levels of both ACTH and COR compared to those transported for 2.5 h or 4 h ($p < 0.05$). In spring, 1 h transport also resulted in significantly elevated COR levels relative to the longer transport durations of 2.5 h and 4 h ($p < 0.05$). While during summer, pigs transported for 2.5 h showed significantly lower serum levels of both ACTH and COR compared to those for 1 h and 4 h transport groups ($p < 0.05$).

Under the same 1 h transport duration, serum ACTH levels were higher in winter than in summer and spring, while winter COR levels were significantly greater than those in spring and summer ($p < 0.05$). When transported for 2.5 h, pigs in spring had significantly lower ACTH levels than those in winter and summer, and their COR levels were also significantly lower than in winter ($p < 0.05$). In the 4 h transport group, summer ACTH levels were significantly higher than in spring and winter, while spring COR levels were significantly lower than those in winter and summer ($p < 0.05$).

These results suggest that among the tested transport durations, 2.5 h of transport induced the least effect on serum ACTH and COR levels. Furthermore, transport during spring had a minimal impact on these hormone levels compared to transport in winter or summer.

3.1.2. Effects of Season and Transport Duration on Serum Enzyme Activities and Creatinine Levels

As shown in Figure 2, during winter, pigs subjected to 2.5 h and 4 h of pre-

slaughter transport exhibited significantly lower serum activities of CK, ALT, and LDH, as well as reduced creatinine (Cr) levels, compared to those transported for 1 h ($p < 0.05$). In spring, 2.5 h of transport resulted in significantly lower CK activity than 4 h of transport, and significantly lower ALT activity compared to 1 h transport ($p < 0.05$). During summer, pigs transported for 2.5 h showed significantly decreased CK activity relative to the 1 h group, lower LDH activity than the 4 h group, and reduced Cr levels compared to both 1 h and 4 h transport ($p < 0.05$).

Under the same 1 h transport duration, all measured parameters—CK, LDH, and ALT activities, as well as Cr levels—were significantly lower in spring compared to both winter and summer ($p < 0.05$). When pigs were transported for 2.5 h, spring again demonstrated significantly lower CK activity compared to both winter and summer, and lower ALT and LDH activities along with Cr levels compared to summer ($p < 0.05$). In the 4 h transport group, spring samples exhibited significantly lower CK and LDH activities and Cr levels compared to summer, and significantly reduced ALT activity compared to both winter and summer ($p < 0.05$).

These results suggest that 2.5 h of pre-slaughter transport had the least impact on ALT, LDH, and CK activities as well as Cr levels. Moreover, transport during spring resulted in minimal changes in these serum enzyme activities and metabolite levels. Compared to 1 h transport, 2.5 h transport duration more effectively mitigated the negative effects of winter transport on enzyme activities and metabolite levels, and partially alleviated the impact of summer transport. While 4 h transport also showed some protective effect relative to 1 h transport in winter, its effect was less pronounced than that of 2.5 h transport.

3.1.3. Effects of Season and Transport Duration on Serum Energy Metabolite Levels

As shown in Table 2, during winter, pigs subjected to 2.5 h of transport exhibited significantly lower serum levels of GLU, LAC, and Glu compared to those transported for 1 h ($p < 0.05$). Additionally, after 4 h of transport, serum GLU levels

were also significantly lower than in the 1 h group ($p < 0.05$). During summer, 2.5 h of transport resulted in significantly lower serum GLU levels compared to 1 h of transport, and significantly lower LAC and Glu levels compared to both the 1 h and 4 h transport groups ($p < 0.05$).

Under the same 1 h transport duration, serum levels of GLU and Glu in spring were significantly lower than those in winter and summer, and LAC levels were significantly lower than in summer ($p < 0.05$). When pigs were transported for 2.5 h or 4 h, serum LAC levels in summer were significantly higher than those in winter and spring ($p < 0.05$).

These results suggest that, in both winter and summer, a pre-slaughter transport duration of 2.5 h was more effective in maintaining serum GLU, LAC, and Glu at significantly lower levels compared to transport durations of 1 h or 4 h.

Table 2. Effects of transport duration on serum energy metabolite levels in different seasons

Parameter	Season	Transport Duration		
		1 h	2.5 h	4 h
GLU levels/ (mmol/L)	winter	5.08±0.36 ^{aB}	3.53±0.15 ^b	4.10±0.27 ^{bc}
	spring	3.70±0.75 ^A	3.60±0.85	4.07±0.27
	summer	5.11±0.98 ^{aB}	4.12±0.22 ^b	4.55±0.54 ^{ab}
LAC levels/ (mmol/L)	winter	4.28±0.53 ^{aA}	2.99±0.58 ^{bA}	3.79±0.48 ^{abA}
	spring	3.62±0.61 ^A	2.83±0.36 ^A	3.37±0.31 ^A
	summer	7.26±1.32 ^{aB}	6.01±0.29 ^{bB}	7.09±2.22 ^{abB}

	winter	0.31±0.02 ^{aB}	0.18±0.53 ^{bAB}	0.22±0.03 ^b
Glu levels/ (mmol/L)	spring	0.18±0.05 ^A	0.14±0.06 ^A	0.20±0.02
	summer	0.29±0.02 ^{aB}	0.22±0.01 ^{bB}	0.24±0.04 ^{ab}

Note: Different lowercase letters within the same row indicate significant differences among groups with different transport durations within the same season ($p < 0.05$). Different uppercase letters within the same column indicate significant differences among groups from different seasons within the same transport duration for a given indicator ($p < 0.05$). The same conventions apply to the subsequent tables.

3.1.4. Effects of Season and Transport Duration on Serum Inorganic Ion Levels

As shown in Figure 3, no significant differences were observed in serum levels of K^+ , Na^+ , or Ca^{2+} among pigs transported for 1 h, 2.5 h, or 4 h within the same season (winter, spring, or summer).

However, significant seasonal variations were found under the same transport duration. When pigs were transported for 1 h, serum K^+ levels in summer were significantly lower than those in winter ($p < 0.05$). Serum Ca^{2+} levels in summer were significantly higher than in spring, which in turn were higher than in winter. Additionally, serum Na^+ levels in summer were significantly elevated compared to both winter and spring ($p < 0.05$).

Under 2.5 h transport, serum K^+ levels in summer remained significantly lower than in both winter and spring, while Na^+ and Ca^{2+} levels were significantly higher than in winter and spring ($p < 0.05$). Similarly, after 4 h of transport, serum Na^+ levels in summer were significantly higher than in winter and spring, and Ca^{2+} levels were significantly higher than in winter ($p < 0.05$).

These results suggest that transport duration (1–4 h) had no significant effect on serum levels of K^+ , Na^+ , or Ca^{2+} within the same season. In contrast, summer was

consistently associated with significantly lower K^+ levels and significantly higher Na^+ and Ca^{2+} levels compared to winter and spring. These trends suggest a potential negative correlation between ambient temperature and K^+ levels, and a positive correlation between temperature and Na^+ and Ca^{2+} levels.

3.1.5. Effects of Season and Transport Duration on Pork pH and Meat Color

As shown in Table 3, during winter, pigs subjected to 2.5 h of transport exhibited a significantly higher $pH_{24\text{ h}}$ in the longissimus dorsi muscle compared to those transported for 1 h or 4 h ($p < 0.05$). Similarly, in summer, 2.5 h of transport resulted in a significantly higher $pH_{24\text{ h}}$ than 1 h of transport ($p < 0.05$).

Under the same 1 h transport duration, both $pH_{45\text{ min}}$ and $pH_{24\text{ h}}$ in spring were significantly higher than those in summer. When transported for 4 h, $pH_{45\text{ min}}$ in spring remained significantly higher than in summer ($p < 0.05$).

Regarding meat color, during both winter and summer, pigs transported for 2.5 h showed significantly lower lightness ($L^*_{45\text{ min}}$) values compared to those transported for 1 h or 4 h ($p < 0.05$). Under the same 1 h transport duration, spring was associated with significantly lower $L^*_{45\text{ min}}$ and significantly higher redness ($a^*_{45\text{ min}}$) and yellowness ($b^*_{45\text{ min}}$) compared to winter and summer. This advantage in color was maintained under 2.5 h transport, with spring showing significantly higher $a^*_{45\text{ min}}$ and $b^*_{45\text{ min}}$ than the other seasons. After 4 h of transport, spring still demonstrated significantly lower $L^*_{45\text{ min}}$ than winter and summer, significantly lower $L^*_{24\text{ h}}$ than summer, and significantly higher $a^*_{45\text{ min}}$, $b^*_{45\text{ min}}$, and $b^*_{24\text{ h}}$ than winter and summer ($p < 0.05$).

These results suggest that a pre-slaughter transport duration of 2.5 h was more effective in maintaining a higher $pH_{24\text{ h}}$ in both winter and summer. Furthermore, transport for 2.5 h during these seasons significantly reduced the increase in lightness (L^*) compared to 1 h or 4 h transport. While transport duration (1-4 h) did not show a significant effect on redness (a^*) or yellowness (b^*), transport during spring

consistently resulted in a more favorable color profile (lower L*, higher a* and b*) and a more moderate pH decline compared to winter and summer.

Table 3. Effects of transport duration on pork pH and meat color in different seasons

Parameter	Season	Transport Duration		
		1 h	2.5 h	4 h
pH _{45 min}	winter	6.03±0.35 ^{AB}	6.17±0.16	6.08±0.11 ^{AB}
	spring	6.47±0.08 ^A	6.57±0.09	6.43±0.14 ^A
	summer	6.05±0.04 ^B	6.29±0.10	5.81±0.10 ^B
pH _{24 h}	winter	5.58±0.12 ^{bAB}	5.88±0.11 ^a	5.59±0.07 ^{bB}
	spring	5.81±0.17 ^A	5.98±0.12	5.84±0.12 ^A
	summer	5.43±0.06 ^{aB}	5.86±0.26 ^b	5.72±0.08 ^{bAB}
L* _{45 min}	winter	46.44±0.74 ^{bB}	43.49±1.02 ^a	47.95±1.98 ^{bB}
	spring	42.59±0.58 ^A	42.62±1.34	43.00±0.65 ^A
	summer	45.82±0.39 ^{bB}	43.53±0.78 ^a	46.42±0.77 ^{bB}
L* _{24 h}	winter	51.68±2.23	48.34±2.08	53.93±2.93 ^{AB}
	spring	49.01±0.68	47.57±0.64	47.87±0.16 ^A
	summer	51.47±0.44 ^{ab}	47.19±1.09 ^a	52.52±0.74 ^{bB}
a* _{45 min}	winter	7.37±0.66 ^B	7.86±0.35 ^B	7.58±0.91 ^B
	spring	9.03±0.68 ^A	9.39±0.61 ^A	9.33±0.67 ^A

	summer	6.97±0.53 ^B	7.36±0.60 ^B	7.54±0.40 ^B
	winter	10.92±0.37	10.25±1.85 ^{AB}	10.53±0.45
a* _{24 h}	spring	11.98±0.86	11.91±0.35 ^A	10.89±0.79
	summer	8.47±0.73	9.30±0.38 ^B	9.40±0.45
	winter	7.65±0.74 ^B	7.78±0.19 ^B	7.09±0.96 ^B
b* _{45 min}	spring	9.85±0.59 ^A	10.74±0.65 ^A	10.04±0.52 ^A
	summer	6.71±0.91 ^B	7.69±0.61 ^B	7.75±0.19 ^B
	winter	9.03±0.50	8.96±0.28	8.05±0.82 ^B
b* _{24 h}	spring	10.47±0.91	11.58±1.03	12.07±0.25 ^A
	summer	7.10±1.87	8.49±0.53	8.84±0.61 ^B

300

301 *3.1.6. Effects of Season and Transport Duration on Pork Texture and Water-Holding*
302 *Capacity*

303 As shown in Table 4, during winter, pigs subjected to 2.5 h of transport exhibited
304 significantly lower cooking loss (%) and drip loss (%) in the longissimus dorsi muscle
305 compared to those transported for 1 h or 4 h ($p < 0.05$). In spring, 2.5 h of transport
306 resulted in significantly reduced cooking loss relative to 1 h transport, and
307 significantly lower drip loss compared to both 1 h and 4 h transport ($p < 0.05$).
308 Similarly, during summer, both cooking loss and drip loss were significantly lower in
309 the 2.5 h transport group than in the 1 h transport group ($p < 0.05$).

310 These results suggest that the water-holding capacity of the longissimus dorsi
311 muscle was significantly poorer in summer compared to winter and spring.
312 Importantly, across all three seasons (winter, spring, and summer), a pre-slaughter

transport duration of 2.5 h was better than 1 h transport in decreasing both cooking loss and drip loss, thereby improving the water-holding capacity of the meat.

Table 4. Effects of transport duration on pork texture and water-holding capacity in different seasons

Parameter	Season	Transport Duration		
		1 h	2.5 h	4 h
Cooking Loss (%)	Winter	25.87±1.62 ^{bA}	17.46±1.83 ^{aB}	23.61±2.94 ^{bB}
	Spring	21.69±3.48 ^{aA}	15.77±0.35 ^{bB}	18.29±1.66 ^{abA}
	Summer	30.42±2.79 ^{aB}	24.55±2.46 ^{bA}	27.40±3.35 ^{abB}
Drip Loss (%)	Winter	4.21±0.35 ^{bB}	2.89±0.46 ^{aB}	3.94±0.44 ^{bA}
	Spring	3.90±0.42 ^{bB}	3.10±0.45 ^{aB}	4.10±0.27 ^{bA}
	Summer	5.23±0.40 ^{bA}	4.22±0.14 ^{aA}	4.68±0.38 ^{abB}
Springiness	Summer	0.65±0.06	0.57±0.05	0.57±0.06
Cohesiveness	Summer	0.72±0.09	0.62±0.04	0.69±0.02
Chewiness (gf)	Summer	115.45±84.46	123.46±45.05	108.01±57.71
Shear Force (gf)	Summer	4049.85±1026.77	3121.30±401.45	2788.28±699.80

3.2. Effects of Season and Lairage Duration on Blood Physicochemical Parameters and Pork Quality

3.2.1. Effects of Season and Lairage Duration on Serum Hormone Levels in Pigs

As shown in Figure 4, during summer, pigs subjected to 16 h of lairage exhibited significantly higher serum ACTH levels compared to those lairaged for 12 h ($p < 0.05$).

Under the same 12 h lairage duration, serum ACTH levels in summer were significantly higher than those in spring. Furthermore, serum COR levels in spring were significantly lower than those in both winter and summer ($p < 0.05$).

Similarly, under the same 16 h lairage duration, serum ACTH levels in summer remained significantly higher than those in winter and spring, while serum COR levels in spring were significantly lower than those in winter and summer ($p < 0.05$).

These results indicate that longer lairage duration (16 h) during summer leads to significantly elevated levels of both ACTH and COR in pig serum, suggesting that extended holding under hot summer conditions may prolong or intensify the stress response rather than allowing for adequate recovery.

3.2.2. Effects of Season and Lairage Duration on Serum Enzyme Activities and Creatinine Levels

As shown in Figure 5, during summer, pigs subjected to 16 h of lairage exhibited significantly higher serum activities of CK, ALT, and LDH, as well as elevated creatinine (Cr) levels, compared to those lairaged for 12 h ($p < 0.05$). In contrast, lairage duration (12 h vs. 16 h) had no significant effect on these parameters during winter or spring.

Under the same 12 h lairage duration, serum CK and ALT activities and Cr levels in summer were significantly higher than those in both spring and winter. Serum LDH activity in summer was also significantly higher than in winter and showed an increasing trend compared to spring ($p < 0.05$). Similarly, under the same 16 h lairage duration, serum CK and LDH activities and Cr levels in summer remained

significantly higher than in winter and spring, while serum ALT activity was significantly elevated compared to both winter and spring ($p < 0.05$).

These results suggest that the impact of extended lairage (16 h) on serum enzyme activities (CK, ALT, LDH) and Cr levels was more pronounced during summer compared to winter and spring. This suggests that holding pigs for a longer duration (16 h) under high summer temperatures may exacerbate stress-related metabolic and tissue damage, rather than providing adequate recovery.

3.2.3. Effects of Season and Lairage Duration on Serum Energy Metabolite Levels

As shown in Table 5, during summer, pigs subjected to 16 h of lairage exhibited significantly higher serum levels of GLU and LAC compared to those lairaged for 12 h ($p < 0.05$).

Under the same 12 h lairage duration, serum LAC levels in spring were significantly lower than those in summer ($p < 0.05$). Similarly, under the same 16 h lairage duration, serum LAC levels in summer remained significantly higher than those in spring ($p < 0.05$).

These results suggest that extended lairage duration (16 h) during summer leads to significantly elevated levels of GLU and LAC in pig serum.

No significant differences were observed in serum Glu levels between the 12 h and 16 h lairage groups across seasons. However, under the same 12 h lairage duration, serum Glu levels in spring were significantly lower than those in summer ($p < 0.05$). This trend persisted under 16 h lairage, with both winter and spring showing significantly lower serum Glu levels compared to summer ($p < 0.05$).

These findings suggest that prolonged lairage (16 h) during summer significantly increases serum GLU and LAC levels. In contrast, increasing lairage duration from 12 h to 16 h did not significantly affect serum Glu levels.

Table 5. Effects of seasonal lairage duration on serum energy metabolite levels

Parameter	Season	Lairage Duration	
		12 h	16 h
GLU levels (mmol/L)	winter	4.35±0.65	4.89±0.48
	spring	3.44±0.79	4.30±0.73
	summer	4.12±0.22 ^a	5.31±0.72 ^b
LAC levels (mmol/L)	winter	3.29±1.14 ^{aB}	7.59±2.41 ^{bAB}
	spring	3.31±0.66 ^B	5.01±0.94 ^B
	summer	7.46±1.70 ^{aA}	11.15±3.79 ^{bA}
Glu levels (mmol/L)	winter	0.20±0.03 ^B	0.23±0.01 ^B
	spring	0.14±0.06 ^B	0.20±0.04 ^B
	summer	0.28±0.05 ^A	0.33±0.03 ^A

3.2.4. Effects of Season and Lairage Duration on Serum Inorganic Ion Levels

As shown in Figure 6, during summer, pigs subjected to 16 h of lairage exhibited significantly higher serum levels of K⁺, Na⁺, and Ca²⁺ compared to those lairaged for 12 h ($p < 0.05$). Under the same 12 h lairage duration, serum K⁺ levels in summer were significantly lower than those in both winter and spring, while serum Na⁺ and Ca²⁺ levels were significantly higher than in winter and spring ($p < 0.05$). Similarly, under the same 16 h lairage duration, serum Na⁺ and Ca²⁺ levels in summer remain significantly higher than those in winter and spring ($p < 0.05$).

These results suggest that the effect of increasing lairage duration from 12 h to 16

h on serum levels of K^+ , Na^+ , and Ca^{2+} was statistically significant and most pronounced specifically during summer, with less impact observed during winter and spring.

3.2.5. Effects of Season and Lairage Duration on Pork pH and Meat Color

As shown in Table 6, during summer, pigs subjected to 16 h of lairage exhibited significantly lower $pH_{45 \text{ min}}$ and $pH_{24 \text{ h}}$ values in the longissimus dorsi muscle compared to those lairaged for 12 h ($p < 0.05$).

Under the same 12 h lairage duration, $pH_{45 \text{ min}}$ in spring was significantly higher than that in winter ($p < 0.05$). When subjected to the same 16 h lairage duration, $pH_{45 \text{ min}}$ in summer was significantly lower than that in both winter and spring ($p < 0.05$).

Regarding meat color, in both spring and summer, pigs lairaged for 12 h showed significantly higher redness ($a^*_{45 \text{ min}}$ and $a^*_{24 \text{ h}}$) values compared to those lairaged for 16 h ($p < 0.05$). During summer, 12 h of lairage also resulted in significantly higher yellowness ($b^*_{24 \text{ h}}$) than 16 h lairage ($p < 0.05$).

Under the same 12 h lairage duration, spring was associated with significantly lower lightness ($L^*_{45 \text{ min}}$ and $L^*_{24 \text{ h}}$) and significantly higher redness ($a^*_{45 \text{ min}}$ and $a^*_{24 \text{ h}}$) compared to both winter and summer ($p < 0.05$). Similarly, under the same 16 h lairage duration, spring demonstrated significantly higher $a^*_{45 \text{ min}}$, $a^*_{24 \text{ h}}$, and $b^*_{45 \text{ min}}$ than winter and summer, while summer showed significantly lower $b^*_{24 \text{ h}}$ than winter and spring ($p < 0.05$).

These results suggest that extending lairage duration to 16 h during summer is more likely to induce a decrease in muscle pH values. Furthermore, 16 h of lairage had a more pronounced effect on meat color parameters compared to 12 h lairage during both spring and summer.

Table 6. Effects of Lairage Duration on Pork pH and Meat Color in different seasons

Parameter	Season	Lairage Duration	
		12 h	16 h
pH _{45 min}	winter	6.21±0.04 ^B	6.14±0.18 ^B
	spring	6.47±0.08 ^A	6.29±0.04 ^B
	summer	6.29±0.10 ^{aAB}	5.73±0.22 ^{bA}
pH _{24 h}	winter	5.64±0.06 ^A	5.55±0.11
	spring	5.82±0.25 ^{AB}	5.60±0.06
	summer	5.88±0.11 ^{aB}	5.58±0.04 ^b
L* _{45 min}	winter	44.46±1.03 ^B	45.28±1.40 ^B
	spring	42.89±0.34 ^A	43.64±0.50 ^A
	summer	45.74±0.44 ^B	46.26±0.30 ^B
L* _{24 h}	winter	50.23±0.66 ^B	51.81±1.05
	spring	47.57±0.64 ^{aA}	51.08±2.79 ^b
	summer	51.27±0.98 ^B	53.05±0.81
a* _{45 min}	winter	10.06±0.66 ^{aB}	8.52±0.83 ^{bB}
	spring	13.02±0.57 ^{aA}	11.51±1.22 ^{bA}
	summer	9.50±0.88 ^{aB}	6.10±0.74 ^{bC}
a* _{24 h}	winter	10.58±0.76 ^B	9.30±0.38 ^B
	spring	15.31±0.61 ^{aA}	12.27±1.25 ^{bA}

	summer	11.33±1.67 ^{aB}	7.42±1.14 ^{bB}
	winter	8.55±0.14	7.83±0.25 ^B
b* _{45 min}	spring	9.62±1.89	9.24±0.71 ^A
	summer	8.86±0.20	7.69±0.61 ^B
	winter	9.26±0.50	8.49±0.53 ^{AB}
b* _{24 h}	spring	10.05±0.75	9.49±0.30 ^A
	summer	9.07±0.59 ^a	7.63±0.99 ^{bB}

407

408 *3.2.6. Effects of Season and Lairage Duration on Pork Texture and Water-Holding*
409 *Capacity*

410 As shown in Table 7, during both spring and summer, pigs subjected to 16 h of
411 lairage exhibited significantly higher cooking loss (%) and drip loss (%) in the
412 longissimus dorsi muscle compared to those lairaged for 12 h ($p < 0.05$).

413 Under the same 12 h lairage duration, cooking loss in summer was significantly
414 higher than in winter, which in turn was higher than in spring. Additionally, drip loss
415 in summer was significantly higher than in spring ($p < 0.05$). Similarly, under the
416 same 16 h lairage duration, cooking loss in summer remained significantly higher
417 than in winter (which was higher than in spring), and drip loss in summer was
418 significantly higher than in both winter and spring ($p < 0.05$). Furthermore, during
419 summer, shear force values were significantly higher in the 16 h lairage group
420 compared to the 12 h group ($p < 0.05$), indicating tougher meat texture.

421 These results suggest that extending the lairage duration from 12 h to 16 h during
422 both spring and summer significantly increases both cooking loss and drip loss while
423 decreasing the water-holding capacity of the longissimus dorsi muscle. Additionally,

the lower shear force values following 12 h of lairage during summer suggest that shorter lairage duration under high-temperature conditions contributes to better pork tenderness and overall eating quality.

Table 7. Effects of lairage duration on pork texture and water-holding capacity in different seasons

Parameter	Season	Lairage Duration	
		12 h	16 h
Cooking Loss (%)	winter	24.45±0.97 ^B	25.03±0.98 ^B
	spring	17.46±1.83 ^{aA}	21.73±1.10 ^{bA}
	summer	30.25±1.13 ^{aC}	34.36±0.56 ^{bC}
Drip Loss (%)	winter	4.32±0.60 ^B	4.59±0.48 ^A
	spring	3.03±0.43 ^{aA}	4.39±0.28 ^{bA}
	summer	4.98±0.94 ^{aB}	6.63±1.03 ^{bB}
Springiness	summer	0.53±0.06	0.57±0.05
Cohesiveness	summer	0.65±0.04	0.62±0.04
Chewiness (gf)	summer	80.71±14.03	123.46±45.05
Shear Force (gf)	summer	4484.21±466.96 ^a	3121.30±401.45 ^b

4. Discussion

When an animal is exposed to external stressors, it activates the hypothalamic-pituitary-adrenal (HPA) axis—the primary endocrine axis responsible for the stress response—leading to the release of hormones such as adrenocorticotrophic hormone (ACTH) and cortisol (COR) to mediate physiological and behavioral adaptations (Lightman et al., 2020, Oyola and Handa, 2017, Sautron et al., 2015). Studies have shown that both short and long durations of pre-slaughter transport significantly exacerbate stress levels in pigs, with particularly pronounced effects under prolonged or extreme conditions (Grandin, 1997, Mota-Rojas et al., 2009, Perez et al., 2002).

The results of this study suggest that during winter, the serum levels of ACTH and COR in the 1 h transport group were significantly higher than those in the 2.5 h and 4 h transport groups. In summer, the 4 h transport group showed significantly elevated serum ACTH compared to the 1 h and 2.5 h groups, whereas the 2.5 h transport group exhibited significantly lower serum COR levels than both the 1 h and 4 h transport groups. These findings suggest that a pre-slaughter transport duration of 2.5 h can more effectively mitigate the increase in stress-related hormone levels compared to 1 h or 4 h transport.

The elevated stress observed during extended transport in summer is likely attributable to high ambient temperatures, which may cause early physical discomfort and accelerated fatigue in pigs. In contrast, the relatively mild and favorable climatic conditions in spring may explain why transport duration had a limited impact on serum hormone levels during this season, as pigs experienced lower baseline stress, and extending transport time provided little additional benefit in reducing stress responses.

After arriving at the slaughterhouse, pigs generally require a lairage period exceeding 12 hours to recover, since procedures such as driving, loading, transport, and unloading induce significant physiological and psychological stress, and the

animals have not returned to a normal state upon arrival. The study by Jama et al.(Jama et al., 2016) demonstrated that pigs slaughtered immediately after transport exhibited significantly higher serum cortisol (COR) levels compared to those allowed a 20-hour rest period post-transport. However, other studies have indicated that excessively prolonged lairage may lead to increased energy depletion due to pre-slaughter fasting and could potentially result in higher mortality rates (Guardia et al., 2004, Guardia et al., 2009, Sutherland et al., 2009).

In the present study, no significant differences were observed in serum ACTH and COR levels between the 12 h and 16 h lairage groups during winter and spring. In contrast, during summer, the 16 h lairage group showed significantly higher serum ACTH levels compared to the 12 h group. This elevation is likely attributable to the elevated thermal stress under high summer temperatures, which may exacerbate the physiological strain on pigs during extended lairage periods.

Serum enzyme activities serve as important indicators of the functional status of corresponding tissues and organs. Under normal physiological conditions, serum activities of enzymes such as CK, LDH, and ALT remain within baseline levels; however, their activities increase when muscle or other tissues are damaged or dysfunctional. Similarly, creatinine (Cr), primarily derived from cardiac and skeletal muscle, is released into the bloodstream only upon muscle injury (Jiaolong et al., 2015, Rafiq et al., 2016, Guo et al., 2019).

In this study, during both winter and summer, pigs subjected to 2.5 h of transport exhibited significantly lower serum enzyme activities and Cr levels compared to those in the 1 h transport group. Furthermore, during summer, the 4 h transport group showed significantly higher LDH activity and Cr levels than the 2.5 h group. These findings may be attributed to the elevated ambient temperatures in summer, which likely accelerated physical exhaustion and energy depletion during prolonged transport.

485 Additionally, during summer, pigs in the 12 h lairage group had significantly
486 lower serum CK, ALT, and LDH activities, as well as Cr levels, compared to those in
487 the 16 h lairage group. It is hypothesized that high temperatures during summer
488 increase the incidence of aggressive behaviors such as biting and fighting, leading to
489 muscle injury. Moreover, extended exposure to elevated temperatures may exacerbate
490 heat stress, further increasing serum enzyme activities and Cr concentrations.

491 Blood glucose and lactate, as critical metabolites in energy metabolism, serve as
492 important indicators of energetic status and stress responses in pigs. During stress,
493 accelerated glycogen degradation and enhanced gluconeogenesis in muscle tissue can
494 lead to elevated blood glucose levels. Furthermore, prolonged stress contributes to
495 increased lactate accumulation in the blood due to anaerobic metabolism (Becerril-
496 Herrera et al., 2010, Mota-Rojas et al., 2012, Pearce et al., 2013).

497 In this study, during both winter and summer, pigs subjected to 2.5 h of transport
498 exhibited significantly lower serum glucose and lactate levels compared to those in
499 the 1 h transport group, suggesting extending transport time to 2.5 h under these
500 seasonal conditions effectively alleviated stress induced by short-distance
501 transportation. In contrast, no significant differences were observed during spring,
502 likely due to the milder temperatures in this season, which resulted in reduced
503 baseline stress levels during short-term transport. These findings are consistent with
504 those reported by Mota-Rojas et al. (Mota-Rojas et al., 2012).

505 Additionally, during summer, pigs in the 12 h lairage group showed significantly
506 lower serum glucose and lactate levels compared to those in the 16 h lairage group,
507 while no notable differences were detected in winter or spring. This phenomenon may
508 be largely attributed to heat stress and increased aggressive behaviors (such as biting
509 and fighting) under high temperatures, which intensify physiological strain and
510 metabolic disturbance when lairage is extended.

511 Glutamate and glutamine are interconvertible and participate in the glutamate-

glutamine cycle. Glutamate also functions as an excitatory neurotransmitter and is a direct precursor of γ -aminobutyric acid (GABA). Several studies have demonstrated that GABA can significantly alleviate stress responses in animals (Balfagon et al., 2022, Bi et al., 2020). Additionally, exogenous supplementation of glutamate and glutamine has been shown to effectively mitigate stress levels (Lian et al., 2018, Salyha and Salyha, 2018, Scharte et al., 2001, Thomas et al., 2022, Wischmeyer et al., 2001). An increase in endogenous glutamate may thus represent a compensatory mechanism to counteract external stress disturbances.

In this study, after 1 h of transport, serum glutamate levels in spring were significantly lower than those in winter and summer, and glutamine levels in spring were significantly lower than in winter. Furthermore, under both winter and summer conditions, pigs subjected to 2.5 h of transport showed significantly lower serum glutamate and glutamine levels compared to those transported for 1 h.

No significant differences were observed in serum levels of inorganic ions among pigs subjected to 1 h, 2.5 h, or 4 h of pre-slaughter transport. This finding is inconsistent with previous reports by Mota-Rojas et al. (Mota-Rojas et al., 2012). The discrepancy may be attributed to the fact that the transport duration in this study did not exceed 4 h, which might have been insufficient to induce significant alterations in ion balance. Conversely, pigs subjected to 16 h of lairage exhibited significantly elevated serum levels of K^+ , Na^+ , and Ca^{2+} compared to those in the 12 h lairage group. This increase may be associated with enhanced heat stress resulting from prolonged lairage under high-temperature conditions. However, due to the complex regulatory mechanisms governing serum ion homeostasis, the exact causes require further investigation.

Meat quality is commonly evaluated through indicators such as pH, color, water-holding capacity, and texture. Among these, pH value directly influences meat color, tenderness, cooking loss, and shelf life (El et al., 2004). In this study, during both winter and summer, pigs subjected to 2.5 h of transport exhibited a significantly

higher pH_{24 h} in the longissimus dorsi muscle compared to those transported for 1 h, indicating that extending transport time to 2.5 h under these seasonal conditions effectively alleviated the decline in pork quality induced by short-term transport stress.

Dur summer, pigs that underwent 16 h of lairage showed significantly lower postmortem pH values than those in the 12 h lairage group. This finding contrasts with the results reported by Driessen et al. (Driessen et al., 2020), who observed that increased lairage time led to higher pH values. The discrepancy may be due to the extended lairage duration adopted in this study.

Furthermore, in both winter and summer, the 2.5 h transport group demonstrated significantly lower lightness ($L^*_{45 \text{ min}}$) values compared to the 1 h and 4 h transport groups, which is consistent with the lower serum LDH activity and LAC levels observed in the 2.5 h group relative to the 1 h group.

Across winter, spring, and summer, the 2.5 h transport group also exhibited significantly lower cooking loss and drip loss than the 1 h transport group, indicating that a 2.5 h transport duration better preserved the water-holding capacity of the meat. In winter, however, the 4 h transport group showed significantly higher cooking and drip losses than the 2.5 h group, likely due to increased discomfort during prolonged transport in cold conditions, leading to reduced water-holding capacity.

Additionally, during spring and summer, the 16 h lairage group had significantly higher cooking and drip losses than the 12 h group, while no significant difference was observed in winter. This may be attributed to reduced physical activity of pigs in cold weather, minimizing further decline in water-holding capacity during lairage.

5. Conclusion

This study investigated stress levels and pork quality in pigs subjected to different transport and lairage durations across seasons. The results demonstrated that spring was associated with lower stress levels and superior pork quality compared to winter and summer.

A transport duration of 2.5 h was found to be more beneficial than 1 h or 4 h, particularly during winter and summer, as it significantly reduced stress responses and improved meat quality. Similarly, a lairage duration of 12 h was more advantageous than 16 h, especially in summer, leading to lower stress levels and better pork quality.

Given that long-distance transportation of pigs has become less common and current practices primarily involve short trips ranging from 0.5 to 4 h, it is recommended to moderately extend transport time within this context when feasible. Furthermore, during summer, it is advisable to reduce lairage duration or enhance cooling measures in holding pens to minimize excessive physical exhaustion caused by fasting and heat stress, thereby preventing deterioration in pork quality.

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586

587 **Data Statement**

588 Original data is available upon request.

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708

Figure Legends

Figure 1. Effects of transport duration on ACTH and Cortisol levels in porcine serum in different seasons

Different lowercase letters above bars indicate significant differences ($p < 0.05$), shared letters indicate no significant difference ($p > 0.05$). The same convention applies to subsequent figures. A: ACTH level; B: COR level.

Figure 2. Effects of transport duration on serum CK, ALT, and LDH enzyme activities and Creatinine levels in pigs in different seasons

A:CK activity; B: ALT activity; C:LDH activity; D:Cr level.

Figure 3. Effects of transport duration on serum inorganic ion K^+ , Na^+ , and Ca^{2+} levels in pigs in different seasons

A: K^+ level; B: Na^+ level; C: Ca^{2+} level.

Figure 4. Effects of lairage duration on serum ACTH and COR levels in pigs in different seasons

A: ACTH level; B: COR level.

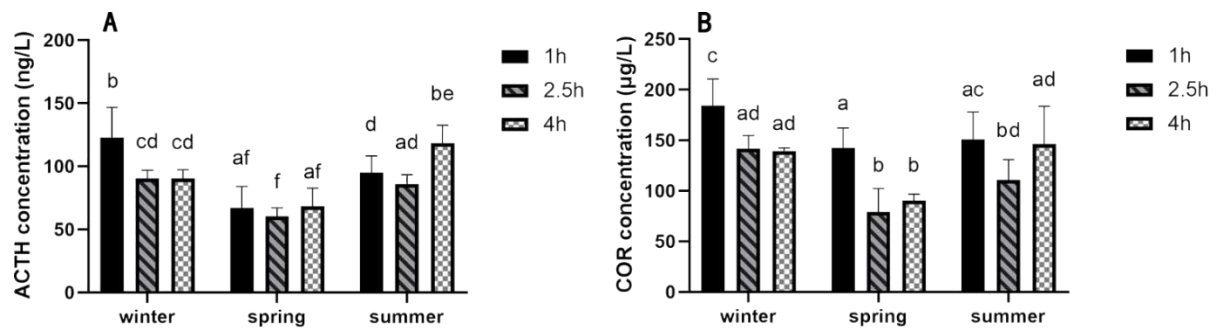
Figure 5. Effects of seasonal lairage duration on serum CK, ALT, and LDH enzyme activities and creatine Levels in pigs

A:CK activity; B: ALT activity; C:LDH activity; D:Cr level.

Figure 6. Effects of lairage duration on serum inorganic ion K^+ , Na^+ , and Ca^{2+} levels in pigs in different seasons

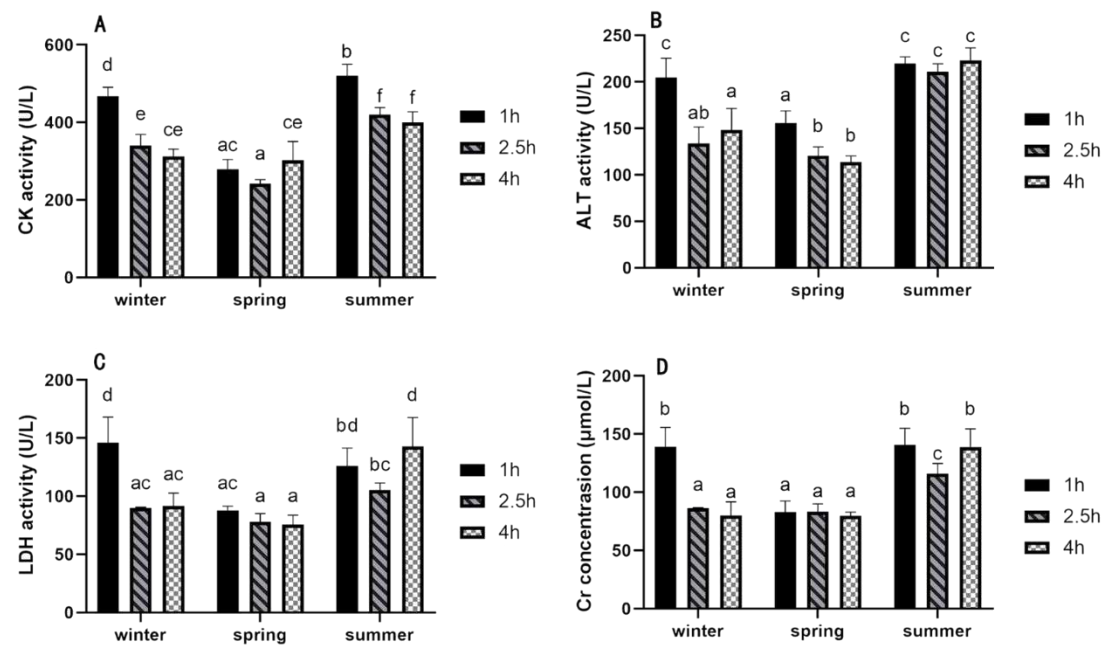
A: K^+ level; B: Na^+ level; C: Ca^{2+} level.

Figure 1



734 Figure 2

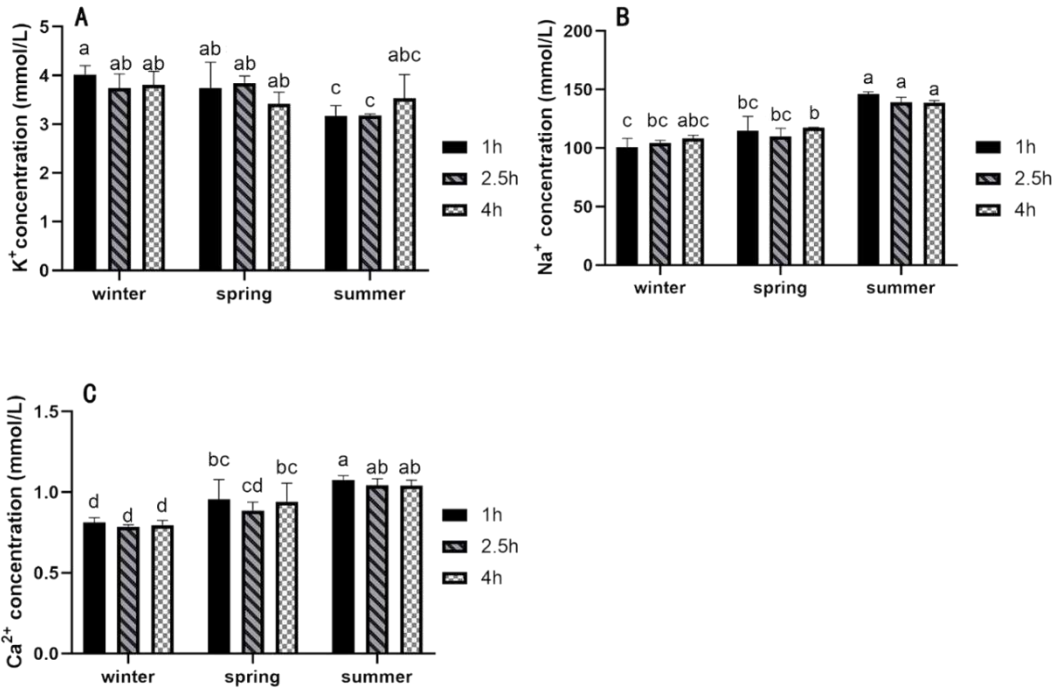
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737 Figure 3

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Figure 4

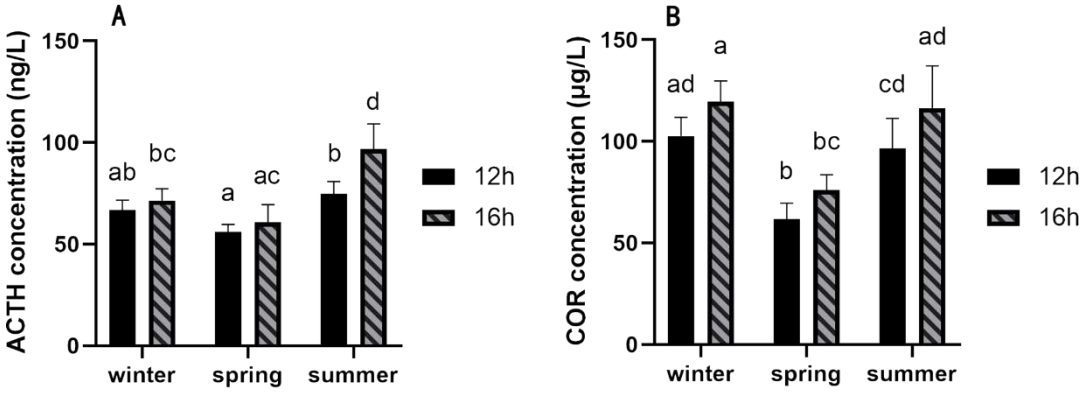


Figure 5

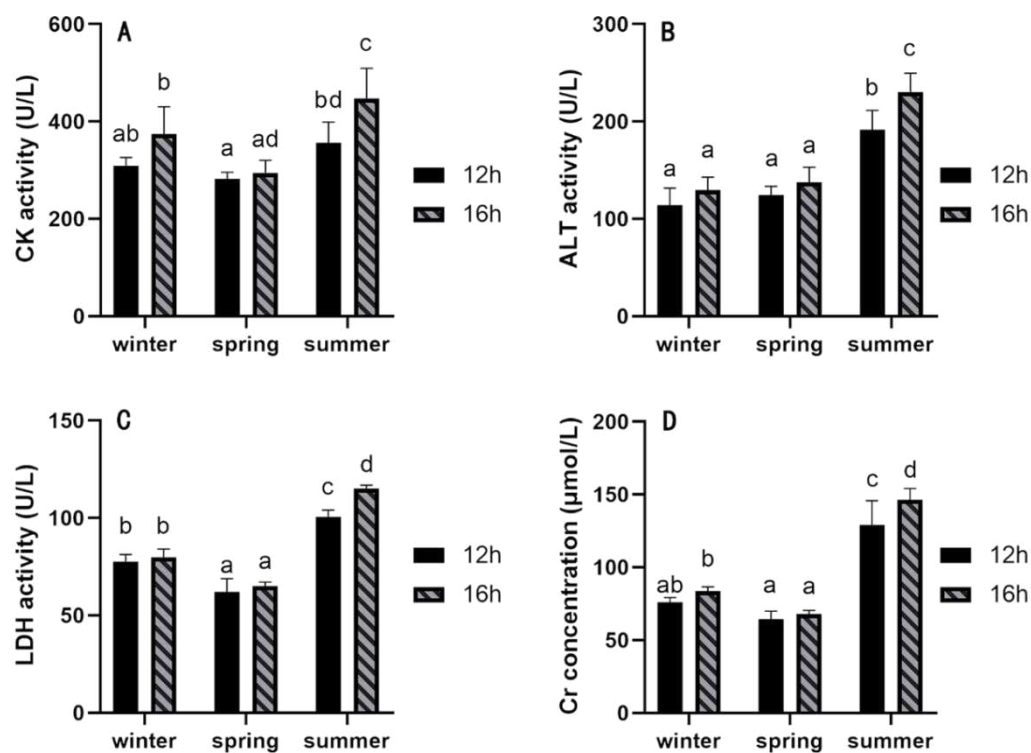


Figure 6

