The interactive effects of transportation and lairage time on welfare indicators, carcass and meat quality traits in slaughter pigs

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Abstract. This study assessed the effects of transportation and lairage time and their interaction on welfare, carcass and meat quality traits in slaughter pigs under commercial conditions. The study was conducted on 120 pigs with a live weight of approximately 115 kg and about six months old. A complete blood picture was measured in pigs to assess pre-slaughter stress. Also, nine different carcass quality parameters including live weight, hot and cold carcass weights, cooling loss, dressing percentage, backfat thickness, meatiness and skin lesions score were measured. The pH and temperature measurements were performed 45 minutes post-mortem. The results showed that short transportation time and slaughtering without lairaging and long transportation time and overnight lairaging negatively influenced the hematological parameters, which meant that the animal welfare was seriously compromised under these pre-slaughter conditions. Long transportation time and overnight lairaging reduced live and carcass weights and increased the incidence of skin lesions on the carcass and DFD pork. In addition, short transportation time and slaughtering without lairaging caused a significant deterioration in pork quality. It can be concluded that, from the standpoint of animal welfare, carcass and meat quality, the above-mentioned pre-slaughter conditions are not recommended to the farmers and/or pork producers.

1. Introduction
Pre-slaughter treatment is the major research topic in animal science since it is closely connected to animal welfare, as well as carcass and meat quality. Assessment of pig welfare status on the day of slaughter is of paramount importance to ensure high pork quality while maintaining the well-being of the animals [1]. On the day of slaughter, several practices have considerable effects on pig welfare and subsequently on carcass and pork quality, such as feeding, slaughter weight, gender, handling during loading or unloading, mixing pigs from different pens, stocking density and time of transportation and lairaging, feed and water withdrawal and the season of slaughter [1-6]. Research findings showed that transportation and lairage time are the two key factors of all pre-slaughter practices that can adversely affect the animal welfare, carcass and pork quality [7-11]. It has been reported that longer transportation (>3 h) results in a higher risk for producing dark, firm and dry (DFD) pork, while pigs subjected to short transportation (<15 min) are more prone to produce pale, soft and exudative (PSE) pork [8]. Furthermore, lairaging shorter than one hour is known to result in a higher occurrence of PSE meat, while when pigs were subjected to overnight lairaging, carcass weight, backfat thickness and
meat temperature were reduced [6]. Also, overnight lairaging poses a higher risk for developing DFD pork and skin lesions on the carcass [11]. Therefore, the aim of this study was to determine the effects of transportation and lairage time and their interaction on welfare indicators, carcass and meat quality traits under commercial conditions.

2. Materials and Methods

2.1. Animals, pre-slaughter handling and slaughter procedure
A total of 120 slaughter pigs with average live weight (LW) of approximately 115 kg and about six months old was evaluated. All the animals were of the same breed (Yorkshire x Landrace crossbreeds) and originated from the same farm. The pigs were raised in a finishing piggery on a completely-slotted floor, in groups of 20 animals per pen with an average stocking density of 1 m² per pig. During the fattening period, food and water were available ad libitum at nipple drinkers and food dispensers. Before transportation, feed and water were not withdrawn. Once loading was finished, the pigs were transported to the same slaughterhouse for less than one hour or more than two hours. Since the information was collected under commercial transport conditions scheduled to pick up animals at several production sites every day, transportation time differed between groups of pigs. Depending on the dynamics of slaughter, the pigs were slaughtered immediately after unloading, or they were held in a lairage overnight and slaughtered the following morning. Lairage density was 0.65 m² per pig. During the period of lairaging, water was provided, but no food. At the end of the lairaging period, the pigs were head-only electrically stunned in batches of six animals without restraining. Following bleeding, the carcasses were processed using conventional practice.

2.2. Welfare indicators
Immediately after the onset of bleeding, blood samples were collected from each pig. They were kept refrigerated (4°C) until being processed immediately on arrival at the laboratory. The vacutainers (2 mL) coated with EDTA were used to measure hematological parameters including white blood cells (WBC), lymphocytes (LYM), middle-sized cells (monocytes, eosinophils, basophils) (MID), neutrophils (NEUT), red blood cells (RBC), red cell distribution width (RDW), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT). The proportions of lymphocytes (LYM%), middle-sized cells (MID%) and neutrophils (NEUT%) were calculated as a percentage of leukocyte concentration on the same device. The indicators of the hematological profile were analyzed by an automatic hematological analyzer Abacus junior vet (Diatron MI PLC, Hungary). Blood glucose (GLUC) levels were measured using a handheld devices analyzer (Gluco Sure Auto Code, ApexBio, Taiwan).

2.3. Carcass and meat quality analyses
The carcasses were weighed immediately after splitting and final washing to obtain the hot carcass weight (HCW), and re-weighed 24 hours after chilling at 4°C to determine the weight of the cooled carcass (CCW). Cooling loss (CL) was calculated based on the difference between the hot and cold carcass weights, expressed as a percentage of the hot carcass weight. The dressing percentage (DP) was calculated as: (hot carcass weight ÷ live weight) x 100. Carcass backfat thickness was measured with a metal ruler at two points (between the 13th and 15th dorsal vertebrae and over M. gluteus medius). Meatiness (in percentages) (M) was calculated according to the Official Gazette [12] based on hot carcass weight and the sum of carcass fat thickness on the back (FTB) and at the sacrum (FTS). Skin lesions (SLC) on the left side of pigs’ carcasses were visually assessed 45 minutes post-mortem according to the Welfare Quality® protocol [13]. The carcasses were divided into the following regions: i) ears; ii) front part of the carcass (from the head to the end of the shoulder); iii) middle part of the carcass (from the end of the shoulder to the rear part of the carcass); iv) rear part of the carcass; and v) limbs (from the accessory digit upwards). Each region of carcass was scored based on a three-
point scale: 0) no visible skin lesions, or only one skin lesion bigger than 2 cm or skin blemishes smaller than 1 cm; 1) between two and 10 skin blemishes bigger than 2 cm; and 2) any wound penetrated into muscles or more than 10 skin blemishes larger than 2 cm. The scoring of the five regions of the carcass was combined into one scoring as follows: 0) all carcass regions with a score of 0; 1) at least one carcass region with a score 1; and 2) at least one carcass region with a score 2. The pH and temperature of the M. longissimus dorsi were measured 45 minutes after slaughter using a pH-meter Testo 205 (Testo AG, Lenzkirch, Germany). Pork quality classes (PSE, normal meat, DFD meat) were determined according to Adzitey and Nurul [14] using pH45 value. The carcasses showing pH45 values lower than 6.0 were classified as PSE meat, while the carcasses showing pH45 values higher than 6.4 were classified as DFD meat. The carcasses with pH45 between 6.0 and 6.4 were classified as normal pork quality.

2.4. Statistical analysis
Statistical analysis of the results was conducted with SPSS software version 23.00 for Windows. The pigs were divided into two groups with regard to transportation time: short transportation (<1 h) and (n=61) and long transportation (>2 h) (n=59). According to lairage time, the pigs were allocated to two groups: the group of pigs slaughtered immediately after unloading (n=60) and the group of pigs slaughtered after overnight lairaging (n=60). Two-way ANOVA with Tukey’s multiple comparison test was performed to test the effect of transportation and lairage time, and their interaction on the welfare indicators, carcass and meat quality traits. Data were described by descriptive statistical parameters as the mean value and pooled standard error of means (SEM). The distribution of pork quality classes in relation to the transportation and lairage time were determined by Chi-square test. A value of P<0.05 was considered significant.

3. Results and Discussions
The effects of transportation and lairage time and their interaction on welfare indicators in slaughter pigs can be seen in Table 1. Two-way interaction (P<0.05) between transportation and lairage time affected red blood cell count and hemoglobin concentration, so that, these parameters increasing markedly when transportation and lairage time decreased. Thus, pigs subjected to short transportation and slaughtered without rest had the highest red blood cell count and hemoglobin concentration (P<0.05). Alterations in red blood cell count, hemoglobin concentrations and hematocrit indicate a situation of adaptation or resistance, in which pigs, after being exposed to the stressful factors, need plenty of oxygen-carrying capacity of blood [2]. Therefore, when pigs encounter stress, the spleen, as a reservoir of red blood cells, contracts and releases erythrocytes into the circulatory system, which provides the muscle mass with a large number of oxygenated erythrocytes, allowing the animal increased physical activity [15-16].

Two-way interaction (P<0.05) between transportation and lairage time was found in white cell parameters, indicating that the number of leukocytes and lymphocytes increased as transportation and lairage time decreased, reaching the maximum values in pigs subjected to short transportation and slaughtered immediately after unloading (Table 1). Lymphocytosis and neutrophilia in stressed animals are associated with changes in leukocyte trafficking and release from the bone marrow after the endogenous secretion of epinephrine or corticosteroids from the adrenal glands [2]. In acutely stressed animals, epinephrine-induced changes are seen within minutes after the release, whereby these effects are characterized by a transient elevation in white blood cells with lymphocytosis [8-9]. In addition, as a result of significant two-way interaction (P<0.05) between transportation and lairage time, the highest blood glucose levels were found in pigs subjected to short transportation and slaughtered without rest (Table 1), which may be explained by catecholamine-mediated glycogenolysis as a response to acute stressors such as loading, transportation, unloading and short lairaging [17-18]. In contrast, when animals are exposed to chronic stress, high white blood cells count with neutrophilia and eosinopenia induced by corticosteroids is evident a few hours after the secretion [8-9]. Therefore, a typical response to corticosteroids with increased number of leukocytes and
neutrophils, as a result of significant two-way interaction ($P<0.05$) between transportation and lairage time, can be seen in pigs subjected to long transportation time and slaughtered after overnight lairaging (Table 1). In the present research, the results of the analyzed welfare parameters suggest that pigs experienced intense acute stress during shorter transport periods and slaughter immediately after unloading. On the other hand, pigs subjected to longer transport periods in combination with prolonged lairage time were under chronic stress. Therefore, it may be argued that in both cases, animal welfare was seriously compromised.

Table 1. Mean values (±pooled SEM) of welfare indicators in slaughter pigs according to transportation time and lairage time ($n=120$)

| Lairage time  | Short transportation time | Long transportation time | Pooled SEM | TT | LT | TT x LT |
|---------------|---------------------------|--------------------------|------------|----|----|---------|
|               | Immediate slaughter       | Overnight slaughter      | Immediate slaughter | Overnight slaughter |
| Number of pigs | 41                        | 20                       | 19         | 40 |
| WBC (10^9/L)  | 23.44a                    | 18.57b                   | 15.87b     | 23.02a |
| LYM (10^9/L)  | 16.83a                    | 12.72b                   | 10.74b     | 13.35b |
| MID (10^9/L)  | 0.22                      | 0.16                     | 0.19       | 0.22 |
| NEUT (10^9/L) | 6.37a                     | 5.70b                    | 4.95b      | 9.45b |
| LYM (%)       | 69.78a                    | 70.14a                   | 68.62ab    | 59.40b |
| MID (%)       | 0.99                      | 0.83                     | 1.27       | 0.98 |
| NEUT (%)      | 27.55a                    | 29.03a                   | 30.11b     | 39.61b |
| RBC (10^{12}/L) | 8.48a                  | 7.04b                    | 7.30b      | 6.92b |
| RDW (%)       | 20.38                     | 20.83                    | 20.24      | 20.79 |
| HGB (g/L)     | 149.07a                   | 136.75b                  | 137.11b    | 129.53b |
| HCT (%)       | 41.63a                    | 40.07                    | 39.55      | 38.39b |
| MCV (fl)      | 49.66                     | 50.15                    | 49.89      | 50.48 |
| MCH (pg)      | 18.05                     | 17.86                    | 18.13      | 17.93 |
| MCHC (g/L)    | 359.90                    | 358.35                   | 360.05     | 360.80 |
| PLT (10^9/L)  | 228.93                    | 264.55                   | 239.95     | 256.70 |
| GLUC (mmol/L) | 9.65a                     | 6.73b                    | 5.87bc     | 4.44c |

TT – significance of transportation time; LT – significance of lairage time; TT x LT – significance of the interaction between transportation and lairage time.

* Statistical significance at ($P<0.05$); NS: not significant ($P>0.05$)
- Different letters in the same row indicate a significant difference at $P<0.05$ ($**$)

The effects of transportation and lairage time and their interaction on carcass and meat quality in slaughter pigs are shown in Table 2. Two-way interaction ($P<0.05$) between transportation and lairage time affected carcass quality traits, indicating that live, hot and cold carcass weights decreased, as transportation and lairage time increased, reaching the minimum values in pigs subjected to long transportation time and slaughtered after overnight lairaging (Table 2). The leading causes of slaughter and carcass weight losses are the decrease of the gastrointestinal tract weight and bladder content during feed and water withdrawal [19]. It has been reported that slaughter weight decreased by 100 g per hour over the 24 h fasting period [20]. Accordingly, it could be considered that the synergistic effect of feed and water deprivation, longer transportation duration and prolonged lairaging increased slaughter weight and carcass loss [19]. Also, pigs after long transportation and overnight lairaging had the highest ($P<0.05$) pH_45 value, skin lesion score and incidence of DFD meat. This can be attributed to the fact that the combined effects of long-distance transportation and prolonged lairaging cause muscle fatigue and breakdown of glycogen, which increases the tendency towards DFD meat [7-11]. Likewise, it is well known that extended lairaging (overnight to >24 h), stimulates fighting behavior and subsequently increasing the occurrence of skin lesions on the carcass [11].

Two-way interaction ($P<0.05$) between transportation and lairage time showed that the lowest pH_45 value, the highest T_45 value and incidence of PSE meat were found in pigs subjected to short transportation time and slaughtered without rest (Table 2). Pigs that underwent short transportation...
and lairaging would not have sufficient time to recuperate from these initial stressors, and, thus, this practice is not recommended, because animals are exhausted and agitated [21-22]. Moreover, such pre-slaughter treatment results in an increase in muscle temperature (+1°C) and lactic acid just prior to slaughter, thus increasing a tendency towards PSE meat [5-11].

Table 2. Mean values (±pooled SEM) of carcass and meat quality traits in slaughter pigs according to transportation and lairage time (n=120)

| Lairage time     | Short transportation time | Long transportation time | Pooled SEM | TT   | LT   | TT x LT |
|------------------|---------------------------|--------------------------|------------|------|------|---------|
|                  | Immediate slaughter       | Overnight slaughter      | Immediate slaughter | Overnight slaughter |       |       |
| Number of pigs   | 41                        | 20                       | 19          | 40   |      |         |
| Carcass quality  |                           |                          |             |      |      |         |
| LW (kg)          | 115.60 a                   | 116.40 a                 | 116.60 b    | 111.40 b | 2.30 | NS   | NS     | *     |
| HCW (kg)         | 94.22 a                    | 95.60 a                  | 95.98 b     | 91.09 b  | 2.04 | NS   | NS     | *     |
| CCW (kg)         | 91.25 a                    | 92.98 a                  | 93.68 b     | 88.34 b  | 2.04 | NS   | NS     | *     |
| CL (%)           | 3.15                       | 2.75                     | 2.37        | 3.02   | 0.51 | NS   | NS     | NS    |
| CD (%)           | 81.49                      | 82.14                    | 82.35       | 81.69  | 0.58 | NS   | NS     | NS    |
| FTB (mm)         | 18.88                      | 16.70                    | 17.95       | 19.50  | 3.13 | NS   | NS     | NS    |
| FTS (mm)         | 36.73                      | 32.85                    | 35.00       | 42.88  | 8.40 | NS   | NS     | NS    |
| M (%)            | 40.00                      | 41.32                    | 40.56       | 38.40  | 2.38 | NS   | NS     | NS    |
| SLC              | 0.71                       | 0.30                     | 0.63        | 1.23   | 0.52 | *    | NS     | *     |
| Meat quality     |                           |                          |             |       |      |         |
| pH45             | 6.02 a                     | 6.21 b                   | 6.21 b      | 6.35 c | 0.07 | *    | *     | *     |
| T45 (°C)         | 40.14                      | 39.36                    | 38.86       | 39.17  | 0.30 | *    | NS     | *     |
| Pork quality classes (%) | 51.22 a | 20.00 b | 21.05 b | 7.50 b | - | - | - | - |
| PSE              | Normal                     | DFD                       |              |       |      |         |
|                 | 48.78                      | 65.00                     | 68.42       | 50.00  | -    | -    | -     | -     |
|                  | 0.00 a                     | 15.00 b                  | 10.53 b     | 42.50 b| -    | -    | -     | -     |

TT – significance of transportation time; LT – significance of lairage time; TT x LT – significance of the interaction between transportation and lairage time. PSE meat: pH45< 6; normal meat: pH45 between 6.0 and 6.4; DFD meat: pH45> 6.4.

* Statistical significance at (P<0.05); NS: not significant (P>0.05)

- Different letters in the same row indicate a significant difference at P<0.05 (**)

4. Conclusion

The results showed that short transportation time and slaughtering without lairaging, as well as long transportation time and overnight lairaging, seriously compromised animal welfare, carcass and pork quality, and, therefore, the above-mentioned pre-slaughter conditions are not recommended to the farmers and/or pork producers.

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