Research in Veterinary Science 193 (2025) 105796

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effects of pre-slaughter logistics duration on stress responses and coping profiles in commercial finishing pigs

Nancy F. Huanca-Marca [a,b], Laura X. Estévez-Moreno [c], Adriana Pastrana-Camacho [a], Matilde Piñeiro [d], Gustavo A. María [a], Genaro C. Miranda-de la Lama [a,*]

[a] Department of Animal Production & Food Science, Agri-Food Institute of Aragon (IA2), University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain
[b] IVITA Station-Maranganí, National University of San Marcos, Lima 106, 08258, Maranganí, Cusco, Peru
[c] Department of Agricultural Sciences and the Environment, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain
[d] Acuvet Biotech, C/Bari 25, 50197 Zaragoza, Spain

ARTICLE INFO

Keywords:
Pig welfare
Pre-slaughter logistics
Stress indicators
Individual differences
Coping styles

ABSTRACT

The present study examined the impact of pre-slaughter logistics duration and coping profiles on physiological indicators of stress and inflammation in commercial finishing pigs. A total of 13 journeys involving 2465 commercial pigs (Hybrid DanBred × Piétrain) were monitored. A subset of 359 pigs was selected for blood sampling at slaughter to assess cortisol, glucose, lactate, creatine kinase (CK), Pig-MAP, and C-reactive protein (CRP). The logistics duration were categorised as follows: short logistics time or ST (< 210 min or <3.5 h), medium logistics time MT (>210–300 min or 3.5–5 h), and long logistics time or LT (>300 - < 420 min or >5 h). The results obtained demonstrate that acute physiological stress responses are associated withST logistics, as evidenced by elevated cortisol and lactate levels. This phenomenon is presumably attributable to inadequate recovery during the period of lairage subsequent to handling and transportation. It has been demonstrated that MT are associated with elevated glucose levels, which may be indicative of a metabolic adaptation to moderate stress. Conversely, LT were associated with elevated CK levels, suggesting the possibility of muscle fatigue or exertion, potentially attributable to protracted logistics processes. Furthermore, the identification of three coping profiles provides additional insight into individual differences in stress responses and offers a potential framework for the early detection of compromised welfare in commercial settings. These findings underscore the need for tailored transport and lairage strategies that integrate both logistical and animal-based factors, particularly resilience.

1. Introduction

The modern pork industry is distinguished by the integration of advanced technologies and efficient logistics systems, which facilitate the efficient transportation, slaughtering and processing of animals. This strategic approach enables the industry to mitigate cost overruns, thereby ensuring its sustained competitive position within the global pork market (Nakrachata-Amon et al., 2024). Concurrently, there is mounting regulatory and public pressure on the industry to guarantee product quality and safety, while also implementing measures to ensure the resilience and sustainability of the business, a process which necessarily includes pig welfare (Sievert et al., 2022). In this context, the pre-slaughter logistics chain encompasses the selection of animals on the farm, fasting, handling, loading, transporting, unloading, housing in lairage pens and finally slaughtering in a licensed slaughterhouse (Miranda-de la Lama et al., 2014). All this requires effective planning, efficient communication, synchronization and optimal allocation of resources between all actors involved, to coordinate these operations efficiently and optimize the time and frequencies of animal delivery to slaughterhouses (Ljungberg et al., 2007). While it has been widely reported that these technological and logistical efforts can improve animal productivity and generate economic benefits for the pork industry, they do not always translate into improvements in animal welfare.

In 2024, the European Union (EU) produced over 23 million tonnes of meat, thus establishing pig production as one of the most economically significant agri-food industries in the Eurozone (Mateos et al., 2024). Within this framework, Spain leads EU pig production, ranking third globally and second in exports, with an annual output of nearly 3

* Corresponding author.
E-mail address: genaro@unizar.es (G.C. Miranda-de la Lama).

https://doi.org/10.1016/j.rvsc.2025.105796
Received 5 November 2024; Received in revised form 15 June 2025; Accepted 30 June 2025
Available online 3 July 2025

million tonnes (MAPA, 2023). To maintain a high level of competitiveness, the national pig sector has adopted a complex vertically integrated system with advanced logistics operations. This system is employed to control all production stages, from breeding to slaughter (Nadal-Roig et al., 2019). These systems have evolved to incorporate automation and control of their processes, in addition to a series of provisions for the regulation of critical points. The propose of these actions is to ensure the uniformity of meat quality and the welfare of the pigs (Huanca-Marca et al., 2025). However, even under optimal logistics, pigs may experience stressors such as fatigue, social conflict, unfamiliar environments, new pathogens, spatial restriction, extreme temperatures, feed and water deprivation, vibration, dust, and road particulates (Miranda-de la Lama et al., 2014; Nakov et al., 2019). Furthermore, it has been demonstrated that a multitude of stressors have the capacity to induce negative mental states such as fear, pain, discomfort, frustration, fatigue, and distress. The expression of such states has the potential to compromise the welfare of the animals and undermine the effectiveness of the production system (EFSA, 2022).

Stress responses are defined as the mental, physiological and behavioural states experienced by animals when confronted with environmental challenges (Colditz, 2022). The assessment of stress involves the utilisation of physiological indicators to measure activity within the hypothalamic-pituitary-adrenal (HPA) axis, either directly or indirectly (Von Borell and Raoult, 2024). Conventional methods for stress response assessment typically involve the measurement of glucocorticoid hormones (e.g. cortisol or corticosterone), catecholamines, glucose levels, lactate (López-Arjona et al., 2020), and plasma creatine kinase activity (Brandt et al., 2015). Furthermore, acute phase proteins such as Pig-MAP and CRP have been identified as potential indicators of poor welfare (Piñeiro et al., 2013). An increase in these indicators is suggestive of a physiological response aimed at restoring homeostasis and protecting the body from pathogens and tissue damage (Saco and Bassols, 2023). Despite the considerable research that has been carried out in the field of applied pig welfare, there are still significant knowledge gaps regarding the impact of logistics chains on the ability of pigs to cope with various handling and management practices that are aimed at optimising processes, particularly in contexts of high technology in modern slaughterhouses (Sardi et al., 2020). This gap is particularly notable given the substantial body of research that has been carried out on the effects of transport on pig welfare (e.g. physiological and behavioural responses) and on specific scenarios in commercial transport practices (e.g. journey distance, truck type) (see EFSA, 2022). Therefore, the present study aims to determine how different durations within the pre-slaughter logistics chain influence physiological stress and inflammatory responses, as well as to explore individual differences in animals' coping profiles within the pre-slaughter environment. We hypothesise that the length of the pre-slaughter logistics chain will differentially affect physiological stress and inflammatory responses in finishing pigs. Additionally, we expect pigs to exhibit different coping profiles that modulate their physiological responses to the novel and aversive conditions of the pre-slaughter logistics chain.

2. Material & methods

The study was conducted in an industrial slaughterhouse situated in the province of Zaragoza (41°53′39 'N, 0°47'17 'W, 293 m.a.s.l.) in the Community of Aragon, Spain, between February and April 2023. The climate of this region is characterised by a dry Mediterranean climate, with an average annual temperature of 15 °C and a rainfall of 317 mm/year, relative humidity of 75 %. The slaughterhouse was found to follow the requirements set forth in the Spanish Official Regulations on animal health, safety, and welfare, as they pertain to the slaughter, processing, storage, import, and export of meat and meat products (RD 993/2014 and EC 853/2004). This slaughterhouse is one of the most modern in the country and has the largest installed capacity, with a potential slaughter capacity of 8000 pigs per day at a rate of 800 animals per hour. The animals involved in this study originated from an integrated system comprising 13 partner farms located in the autonomous communities of *Castilla y León*, *Navarra*, and *Aragón*. This integrated system utilises a standardized genetic line, along with uniform husbandry, management and feeding practices. In accordance with this system, the pre-slaughter logistic chain is responsible for planning the weights and homogeneous batches of animals to be transported and slaughtered. All animals were provided with free access to water during the transportation and lairage process and were transported and slaughtered in accordance with the European Union's welfare regulations (EC 1/2005).

2.1. Study description

The evaluation of the pre-slaughter logistics chain was conducted as a cross-sectional study with the objective of assessing the physiological profile of slaughtered commercial pigs (Hybrid DanBred [sows] × Piétrain [boars]) with an average live weight of 128.12 ± 16.11 kg. In total, 13 journeys transporting 2465 pigs (1213 males and 1252 females) were monitored. For each journey studied, the first 27 to 28 pigs that were stunned and entered the processing chain for bleeding ($n = 359$) were sampled. This sampling strategy was systematic and consecutive, following the natural sequence of the slaughter process. While the selection was not strictly random, it allowed for operational feasibility and ensured animal traceability. Moreover, we consider this approach methodologically acceptable, as pigs from different truck compartments and levels were mixed in a common lairage pen prior to stunning, which reduces the risk of systematic bias within each lot. In accordance with the company's standard procedures and those of its integrated farms, all animals were required to undergo a 12-h fasting period prior to loading and transport to the slaughterhouse. During this period, the animals were kept in pre-transport pens, where they were supplied with water ad libitum. The loading of the animals was conducted in an area adjacent to the pre-transport pens, with the pigs being guided through aisles to the loading ramp of the truck. According to the company's manual of good practices, the loading time is stipulated to be between 50 and 90 min.

2.1.1. Transport operations

The duration of the transportation of the pigs ranged from 20 to 243 min, thus complying with the General Transport Regulation (EC 1/2005), which sets a maximum limit of 24 h for the transportation of adult pigs. According to the company's logistics planning reports, 60 % (±10) of the journey was undertaken on highways, 25 % (±10) on secondary roads, and 15 % (±6) on unpaved rural roads. It was observed that journeys from more distant origins commenced between 4 and 5 am, while those from closer origins began between 6 and 7 am. The internal truck temperature was recorded at 16.3 °C (±3.7). The trucks used in this study were trucks (Scania Södertälje©, Sweden or MAN©, Munich, Germany) with trailers (Carrozzeria Pezzaioli©, Montichiari, Italy or Carrocerria MASER©, Lleida, Spain). These trailers have three floors with six compartments per floor (each compartment was 210 cm long × 264 cm wide and 84 cm high, giving a total surface area of 5.54 m^2 per compartment and an average rearing density of 0.42 m^2 /pig). The total load capacity of the trucks was about 27,000 kg. The trailer was equipped with an automatic drinking system and a water tank (>250 kg) to supply water during the journey. It also had mechanical and passive ventilation on each floor and in the compartments. Forced ventilation system with 6 fans per floor, each fan had a diameter of 225 mm and a flow rate of 11,700 m2/h according to EC 1/2005; and for natural ventilation it had side vents with sliding panels. The trailer had a hydraulically controlled loading and unloading lift with a lifting capacity of 1500 kg and had non-slip floors with built-in side protection.

2.1.2. Pre-slaughter and slaughter operations

The slaughterhouse is operational from Monday to Saturday, with operations commencing at 05:00 and concluding at 23:00. Upon arrival at the slaughterhouse, the pigs were unloaded into a roofed unloading bay (54 m^2) using the hydraulic platform of each truck. Once the entirety of the batch had been unloaded, the animals were weighed in groups on the unloading dock via an automated weighing apparatus. The duration of the unloading process ranged from 19 to 72 min. Following unloading, the animals were led through the aisles to a lairage area comprising 30 pens. They were then introduced to their assigned pen (30x4m) and rested for a period of 0.4 to 5 h after arrival at the slaughterhouse. All animals from the same truck shared the same holding pen. During their stay in the pens, all pigs were showered using a sprinkler system and provided with unlimited access to water via stainless steel drip troughs.

Subsequently, the pigs were conveyed from the lairage area to the stunning area via a series of straight aisles with non-slip flooring. The pigs were led with the assistance of a trained operator, utilising a pig-storing panel, to prevent any harm being caused to the animals. Upon reaching the stunning area, the animals were directed through five sluices, equipped with movable gates, in a safe and controlled manner. In the side airlock to the stunning system, the animals were directed sideways into the gondola via automatic gates. The slaughterhouse is equipped with two stunning towers, each with a capacity of six gondolas and a capacity of eight animals per gondola. The pigs were subjected to a 90 % CO_2 chamber stun for approximately 60 s in a gondola immersion elevation system (MECO-26, NORIA-MECANOVA©, Toledo, Spain). Following stunning, the pigs descended a gradual slope to an automated conveyor belt, where each animal was lifted and incorporated into the chain to be led to the bleeding area. In this area, the animals were manually bled with a midline neck cut (a deep cut of the blood vessels leaving the heart) in an upright position by four trained operators. The total duration for pre-slaughter logistics chain, from the moment the animals left the farm until the commencement of the slaughter process, exhibited a range of 48 to 408 min.

2.2. Physiological stress indicators

At the time of bleeding, 4.5 ml of blood was collected from each pig using tubes (Vacuette® CAT, Kremsmünster, Austria) containing a clot activator for the purpose of serum extraction. Once all samples had been collected from each farm, they were stored at 4 °C in a refrigerated environment. Subsequently, the samples were subjected to centrifugation at 1300 ×*g* for a period of 10 min, with the objective of obtaining serum and plasma. The serum samples were analysed for a range of parameters, including cortisol, glucose, lactate, creatine kinase, and acute-phase proteins such as Pig-MAP and C-reactive protein. Suerum cortisol levels were quantified using the amplified chemiluminescence technique with an automated chemiluminescence immunoassay analyser (IMMULITE 1000, Immunoassay System©). Serum glucose levels were determined by the enzymatic-colorimetric method, employing an automated clinical chemistry analyser (Biosystems BA400, Barcelona, Spain). Serum lactate and CK levels were determined by the enzymatic method using an automated clinical chemistry analyser (Biosystems BA400, Barcelona, Spain). The concentration of Pig-MAP was determined by sandwich ELISA (Acuvet ELISA PigMAP, Acuvet Biotech©, Zaragoza, Spain) which is based on two anti-Pig-MAP monoclonal antibodies and calibrated in accordance with the European standard for porcine acute phase proteins (Piñeiro et al., 2009). The assay demonstrated a detection limit of 0.15 μg/ml, accompanied by inter- and intra-assay coefficients of variation (CV) of <10 %. The concentration of CRP was determined by immunoturbidimetry, using a porcine-specific assay (Turbovet pig CRP, Acuvet Biotech©, Zaragoza, Spain).

2.3. Statistical analyses

A correlation matrix was constructed to identify potential associations between the various physiological indicators, using the Spearman's correlation test ($p < 0.05$). The associations between the time spent on the processes involved in pre-slaughter logistics (transport time, unloading time, lairage, and total pre-slaughter logistics time) and physiological stress indicators were examined using multifactorial two-way models. The collinearity of the variables included in each model was verified by calculating the adjusted GVIF (GVIF1/(2 × df)), as proposed by Fox and Monette (1992), which is suitable for categorical variables with multiple degrees of freedom. It was verified that the resulting values were < 5. The parametric nature of the variables was assessed using the Shapiro–Wilk test, considering $p \geq 0.05$ as indicative of normal distribution. The General Linear Model (GLM) procedure was used when the dependent variable (the physiological stress indicators) followed a parametric distribution, and the assumption of homoscedasticity was met. Homoscedasticity was assessed using Levene's test, and considered present when $p > 0.05$, indicating equal variances across groups. For variables that did not meet the assumptions of normality and/or homoscedasticity, logarithmic and Box–Cox transformations were initially applied, and model assumptions to perform General Linear Models (GLMs) were assessed. In parallel, Generalized Linear Models (GZLMs) were fitted using gamma distributions, which are appropriate for continuous, strictly positive, and right-skewed data. Model performance of all models was evaluated through the comparison of the Information Criterion (AIC) values (Anderson et al., 2012; Celis and Labrada, 2014). In all cases, gamma-based models consistently yielded the best fit as had the lowest AIC values. As a result, GZLMs with a gamma distribution and log link function were selected for Cortisol, Lactate, CRP, CK, and Pig MAP.

For each physiological variable, four separate two-way models were performed. In each model, one of the pre-slaughter logistic time variables (total pre-slaughter logistics time, transport time, unloading time, or lairage time) was included as a fixed effect, along with sex and the interaction between time and sex. Based on the pre-slaughter logistic conditions under which this study was conducted, total pre-slaughter logistics time (time elapsed from leaving the farm to the commencement of slaughter) was categorised into three distinct durations periods: short time or ST (< 210 min or <3.5 h), medium time or MT (>210–300 min or 3.5–5 h) and long time or LT (>300- < 420 min or >5 h). Transport time had different categories TT1 (<18 min), TT2 (60–132 min),TT3 (>210 min). Unloading time had two categories: short or U1 (<34 min) and long or U2 (>40 min), and lairage had also two durations, named L1(<132 min) and L2 (>132 min). Furthermore, the relationships between transport time, unloading time and stress indicators were examined jointly using a two-way model with a fixed effect of transport time, a fixed effect of unloading time, and an interaction effect between transport time and unloading time. Value of $p < 0.05$ was considered statistically significant. The analyses were performed using SPSS version 29. For the calculation of adjusted GVIF values, the *car* package in RStudio was used.

Furthermore, profiles of pigs according to their physiological indicators of stress (cortisol, lactate and glucose) were identified by hierarchical cluster analysis (using Ward's method and squared Euclidean distance). The number of clusters was determined through the visualisation and interpretation of the dendrogram. A new variable was constructed to identify each pig according to its cluster number of membership. Subsequently, significant associations between cluster membership and blood variables (cortisol, lactate, glucose, CK, Pig-Map and C-reactive protein) and logistic variables (total pre-slaughter logistics time, transport time, unloading, lairage), sex and live weight were analysed. The Kruskal-Wallis or ANOVA tests were employed for continuous variables, while the Chi-square or Likelihood Ratio test was used for qualitative variables ($p < 0.05$) (Celis and Labrada, 2014). All analyses were conducted using the statistical software package SPSS® Statistics 29.

3. Results

The study revealed no mortality among the 2465 animals comprising the 13 groups. However, a prevalence of 0.40 % ($n = 10$) of nonambulatory animals was documented.

3.1. Physiological conditions at slaughter

The mean physiological indicators of stress are shown in Table 1. As demonstrated in Table 2, a significant correlation exists between the various physiological indicators. A positive correlation was observed between cortisol and lactate concentration ($p < 0.001$), while a negative correlation was evident between cortisol and glucose ($p < 0.001$). Furthermore, a positive correlation was observed between lactate concentration and Pig-Map ($p < 0.01$) and CRP ($p < 0.001$).

3.2. Pre-slaughter logistic chain effects

Table 3 presents the concentration levels of cortisol ($p < 0.01$), lactate ($p < 0.001$), CK ($p < 0.001$), glucose ($p < 0.01$), CRP ($p < 0.01$) and Pig-MAP ($p > 0.05$) with respect to the total pre-slaughter logistics time, categorised as short (ST), medium (MT) and long (LT). A significant increase in cortisol and lactate concentration was observed during the total logistic in ST group. However, a higher glucose concentration was observed in animals that underwent in MT group, and no significant differences were identified between ST and LT. Furthermore, pigs that remained in the logistics process for a longer time (LT) exhibited elevated CK levels. In the case of CRP levels differences were between MT and LT groups. No significant differences were found in Pig-Map concentration across the three logistic time points. Sex, along with the interaction between total pre-slaughter logistics time and sex, did not influence the physiological stress indicators in pigs.

The values of physiological stress indicators are displayed in Table 4 according to transport time categories. A significant effect of short transport time (TT1) on cortisol and lactate concentration was observed ($p < 0.01$). Similarly, the glucose concentration was observed to be diminished in animals that had TT1 ($p < 0.05$), whereas the CK levels increased significantly as transport time got longer ($p < 0.001$). No significant associations were identified between the concentration of Pig-Map and CRP and transport time ($p > 0.05$). Sex, and its interaction with transport time categories had no effect on the physiological indicators of stress in pigs ($p > 0.05$). No significant differences were observed in the concentration of cortisol, lactate, glucose, CK, Pig-Map and CRP according to lairage time ($p > 0.05$).

Significant interactions between transport time (TT) and unloading time (U) were observed for five of the six physiological indicators evaluated (Table 5). Notably, the group with the shortest transport and unloading times (TT1–U1) exhibited the highest cortisol concentrations and the lowest glucose levels ($p < 0.001$). For cortisol, values in this group were significantly higher than in any other, while for glucose, they were significantly lower than those in the TT2–U2 and TT3–U1 groups, which showed the highest levels. In the case of CK ($p < 0.05$), an increasing trend was observed with longer transport durations, especially when combined with prolonged unloading. Pig-Map levels were significantly lower in the group with the combination of the longest transport and unloading time (TT3–U2) ($p < 0.05$). Regarding CRP ($p < 0.001$), the highest values were again recorded in the TT1–U1 group, whereas the lowest were found in TT3–U2 (prolonged transport and unloading).

Table 1
Means (± S.D.) of physiological stress indicators in slaughter pigs.

Physiological stress indicators	n	Mean	Mínimum	Máximum
Cortisol (nmol/L)	359	183.42 ± 81.04	30.9	541.0
Lactate (mmol/L)	359	13.93 ± 4.21	6.9	33.2
Glucose (mg/dl)	359	234.49 ± 71.64	79.0	426.0
CK (U.I./L)	359	4313.64 ± 2510.09	668.0	11,509.0
Pig-Map (mg/ml)	359	0.98 ± 0.68	0.2	7.77
CRP (mg/ml)	358	0.027 ± 0.027	0.002	0.174

CK: Creatine Kinase; CRP: C-Reactive protein.

3.3. Coping profiles

The hierarchical cluster analysis indicated the presence of three clusters or profiles (conglomerate 1, 2, 3), which were determined by three indicators (cortisol, lactate and glucose) of animal welfare on physiological response. The distribution of animals within each cluster was not homogeneous, with cluster 1 or high stress pigs (HS) comprising 92 pigs, cluster 2 or intermediate stress pigs (IS) comprising 142 pigs, and cluster 3 or low stress pigs (LS) comprising 124 pigs. The concentration of cortisol, lactate and CK was found to be higher in HS pigs (Fig. 1). Furthermore, the mean glucose concentration was found to be higher in the HS pigs than in the other groups. Pigs in the intermediate stress category are distinguished by lower cortisol and lactate levels, although they exhibit higher glucose concentrations. LS pigs are distinguished by lower cortisol, lactate, glucose, CK and higher Pig-Mag concentrations, indicating reduced stress levels. Significant statistical differences were identified between the HS, IS and LS pigs for the physiological indicators of stress, namely cortisol, lactate and glucose ($p < 0.001$). However, no statistically significant differences were observed between the groups for the levels of CK, Pig-Map and CRP ($p > 0.05$).

Table 6 illustrates the profiles of the pigs and their suitability for the pre-slaughter logistics to which they were exposed. A short time spent within the logistic chain was identified as a significant factor in the observed outcomes of HS pigs (55.4 %; $p < 0.01$). Nevertheless, a medium time span was found to have a statistically significant impact on the IS pigs ($p < 0.01$). Conversely, a longer period spent within the logistics chain was found to have a significant impact on LS pigs (70.8 %; $p < 0.01$). A shorter transport time had a significant impact on HS pigs (44.6 %; $p < 0.001$), with a higher percentage of these pigs being observed during TT1 transport (54.2 % and 52.8 %). Moreover, the longer the transport time (TT3), the lower the impact on HS pigs ($p < 0.001$), while a higher percentage of IS and LS pigs were observed (30.3 % and 26.6 %, respectively). The unloading time (U1) had a greater impact on HS and IS pigs (69.6 % and 51.4 %, respectively; $p < 0.05$). However, no statistically significant differences were observed for lairage time or sex in the three groups of pigs according to their blood profile (0.05). Conversely, the LS group of pigs exhibited a higher live weight (16.4 %; $p < 0.001$).

4. Discussion

The findings of this study demonstrate that, even within controlled environments, a logistics chain of brief duration (<210 min) can exert a substantial influence on the stress response of pigs. This phenomenon can be attributed to the presence of stressful processes experienced by the animals in a remarkably short time frame, which does not allow for recovery during the lairage period. It is important to note that these results are based on the study of a logistics chain that includes animals of similar weight and genetic line (Hybrid DanBred × Piétrain), as well as the standardized processes of fasting, loading/unloading, handling stalling, stunning and bleeding. Moreover, the present study also demonstrated that pigs adopt divergent coping physiological strategies when confronted with a novel challenge, such as the pre-slaughter logistic chain. This finding has practical implications for slaughterhouse operations and theoretical ramifications for the study of individual differences. The demonstration that such profiles reflect divergent physiological strategies in the face of the same challenge (Kanitz et al., 2019) enriches theoretical understanding. In summary, this study provides novel evidence that will contribute to the refinement of operations in modern pre-slaughter logistics chains.

Table 2
Correlations (r) between physiological stress indicators in slaughter pigs.

Physiological stress indicators	Cortisol (nmol/L)	Lactate (mmol/L)	Glucose (mg/dl)	CK (U.I./L)	Pig-MAP (mg/ml)	CRP (mg/ml)
Cortisol (nmol/L)		0.267***	−0.209***	0.066	−0.076	0.052
Lactate (mmol/L)			0.132*	0.1	0.143**	0.206***
Glucose (mg/dl				−0.012	0.034	0.066
CK (U.I./L)					−0.047	0.038
Pig-MAP (mg/ml)						0.338***
CRP (mg/ml)						

Significance differences at *$p < 0.05$. ** $p < 0.01$ *** $p < 0.001$ according to the Spearman test. CK: Creatine Kinase; CRP: C-Reactive protein.

Table 3
Adjusted means (± S.E.) of physiological stress indicators according to the total pre-slaughter time.

Physiological stress indicators	Short time ST (<210 min)	Medium time MT (>210 min < 300 min)	Long time LT (>300 min < 420 min)
Cortisol (nmol/L)**	197.72 ± 6.86 ^{a}	178.87 ± 7.81^{ab}	166.53 ± 6.34 ^{b}
Lactate (mmol/L)***	15.21 ± 0.34 ^{a}	13.18 ± 0.38 ^{b}	12.90 ± 0.32 ^{b}
Glucose (mg/dl)** £	225.93 ± 5.79 ^{a}	256.26 ± 7.45 ^{b}	228.82 ± 6.46 ^{a}
CK (U.I./L)***	3322.84 ± 143.69 ^{a}	4274.46 ± 232.64 ^{b}	5526.82 ± 262.29 ^{c}
Pig-Map (mg/ml)	0.95 ± 0.04 ^{a}	1.07 ± 0.05 ^{a}	0.93 ± 0.04 ^{a}
CRP (mg/ml)**	0.027 ± 0.002 ^{ab}	0.033 ± 0.003 ^{a}	0.022 ± 0.002 ^{b}

Significance differences at *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. CK: Creatine kinase; CRP: C-Reactive protein. Min: Minutes. Least Square Means (± S.E.) are reported for GLM£ and Estimated Marginal Means (± S.E.) for GZLM-gamma. Different letters (a.b.c) in the same row indicate post hoc significant differences.

Table 4
Adjusted means (± S.E.) of the physiological stress indicators according to three different transport times.

Physiological stress indicators	TT1 (<18 min)	TT2 (60-132 min)	TT3 (>210 min)
Cortisol (nmol/L)**	216.10 ± 9.45 ^{a}	176.38 ± 5.37 ^{b}	162.28 ± 6.93 ^{b}
Lactate (mmol/L)**	15.20 ± 0.45^{a}	13.58 ± 0.28 ^{b}	13.31 ± 0.39 ^{b}
Glucose (mg/dl) *£	213.65 ± 7.50 ^{a}	238.77 ± 5.27 ^{b}	246.57 ± 7.46 ^{b}
CK (U.I./L)***	3135.65 ± 180.37 ^{a}	4456.37 ± 178.53 ^{b}	5107.26 ± 287.06 ^{c}
Pig-Map (mg/ml)	0.99 ± 0.05 ^{a}	1.01 ± 0.04 ^{a}	0.89 ± 0.04 ^{a}
CRP (mg/ml)	0.029 ± 0.002 ^{a}	0.028 ± 0.002 ^{a}	0.021 ± 0.01 ^{a}

Significance differences at *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. TT1: Short time; TT2: Medium time; TT3: Long time. CK: Creatine kinase; CRP: C-Reactive protein. Min: Minutes. Square Means (± S.E.) are reported for GLM£ and Estimated Marginal Means (± S.E.) for GZLM-gamma. Different letters (a.b.c) in the same row indicate post hoc significant differences.

4.1. Physiological conditions at slaughter

The analysis of physiological stress response variables offers a valuable insight into the ways in which an organism responds to different stressors in diverse settings and contexts (Koolhaas et al., 1999). In the present study, most animals exhibited cortisol levels above the species-specific basal range (>166 nmol/L; Rey-Salgueiro et al., 2018). Prior research indicates that elevated cortisol levels are indicative of heightened stress or exertion during pre-slaughter handling (Machado et al., 2022; Acevedo-Giraldo et al., 2020). Furthermore, the lactate level was found to be higher than the previously established basal levels (3.6–4 mmol/L) (Mota-Rojas et al., 2012; Edwards et al., 2010). This increase may be indicative of both the physical exertion of the animals and their emotional stress response. Glucose levels were observed to be higher than the basal range (75–150 mg/dl), a finding that aligns with reports from previous transport studies by various authors (Acevedo-Giraldo et al., 2020; Mota-Rojas et al., 2012). The elevation in glucose levels may be indicative of an adaptive metabolic response during the pre-slaughter handling process. Additionally, CK exhibited higher than basal levels of the species (830–954 IU/L) (Aboagye, and Dall'Olio, S., Tassone, F., Zappaterra, M., Carpino, S., Nanni Costa, L., 2018). The elevation in CK levels is primarily attributable to muscle damage resulting from overcrowding, abrupt vehicle movements, and extended transportation periods, which can precipitate muscle damage (Brandt et al., 2015).

A key aspect to consider is the possible effect of pre-slaughter fasting on the stress response of pigs. In the pork industry, this period is usually extended to between 12 and 18 h (with access to water) to reduce the risk of faecal contamination of the carcass, stabilise the post-mortem pH of the meat and minimise the incidence of vomiting, diarrhoea and gastric torsion during the pre-slaughter stages (Driessen et al., 2020). In our study, a standard 12-h fast did not affect cortisol, lactate, creatine kinase (CK), and glucose concentrations, as the total exercise and logistical period did not exceed 17 h. This time falls within the recommended fasting range of 12–24 h (Faucitano et al., 2010; Dalla-Costa et al., 2019; Driessen et al., 2020). Several studies have examined the impact of extended fasting on fattening pigs, Faucitano et al. (2006) found no significant differences in cortisol concentration in pigs subjected to 4- and 24-h fasts. Similarly, Dalla-Costa et al. (2016) observed no significant variations in CK and lactate levels in animals fasted for 24

Table 5
Least square and estimated marginal means (±S.E.) of stress response values as a function of three different transport times and two different unloading times.

Physiological stress indicators	TT1 (<18 min)		TT2 (60-132 min)		TT3 (>210 min)		Effect (p-value)		
	U1 (<34 min)	U2 (>40 min)	U1 (<34 min)	U2 (>40 min)	U1(<34 min)	U2(>40 min)	TT	U	TT*U
Cortisol (nmol/L)	250.85 ± 12.83^{a}	152.14 ± 10.92^{b}	177.28 ± 7.34^{b}	174.72 ± 7.23^{b}	155.18 ± 7.87^{b}	183.04 ± 13.13^{b}	*	*	***
Lactate (mmol/L)	15.12 ± 0.55^{a}	15.24 ± 0.77^{a}	14.03 ± 0.41^{b}	13.18 ± 0.38^{b}	13.79 ± 0.49^{b}	12.49 ± 0.63^{b}	**	ns	ns
Glucose (mg/dl)£	194.75 ± 9.00^{a}	250.83 ± 12.62^{b}	232.72 ± 7.28^{b}	244.82 ± 7.28^{b}	259.00 ± 8.92^{b}	221.70 ± 12.62^{b}	ns	ns	***
CK (U.I./L)	2973.64 ± 203.67^{a}	3610.03 ± 346.75^{ab}	3967.23 ± 220.01^{bc}	4981.62 ± 276.25^{cd}	5292.37 ± 359.45^{d}	4730.43 ± 454.36^{cd}	***	ns	*
Pig-Map (mg/ml)	0.89 ± 0.55^{ab}	1.18 ± 0.10^{a}	0.93 ± 0.05^{a}	1.06 ± 0.05^{a}	1.00 ± 0.06^{a}	0.69 ± 0.12^{b}	**	ns	*
CRP (mg/ml)	0.035 ± 0.003^{a}	0.02 ± 0.005^{b}	0.024 ± 0.002^{b}	0.033 ± 0.003^{a}	0.023 ± 0.03^{b}	0.017 ± 0.002^{b}	*	*	***

Significance differences at *$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$*. ns: not significant ($p > 0.05$). CK: Creatine kinase; CRP: C-Reactive protein; min: Minutes. U: Unloading; TT: Transport time. Least Square Means (± S.E.) are reported for GLM£ and Estimated Marginal Means (± S.E.) for GZLM-gamma.

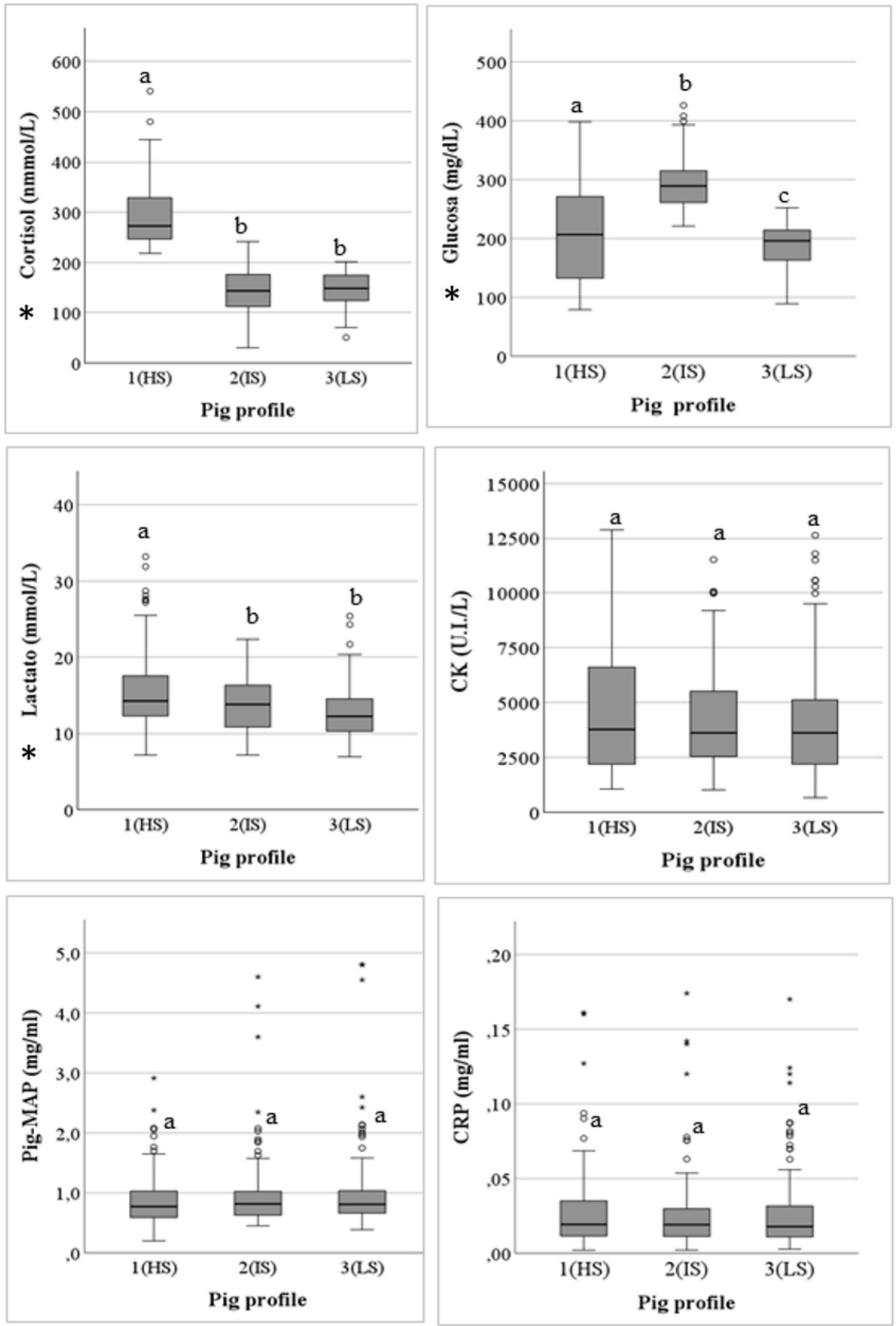

Fig. 1. Stress coping styles in commercial finishing pigs. Different letters (a, b, c) indicate significant differences among the conglomerate, based on Bonferroni-adjusted pairwise comparisons following a Kruskal-Wallis test ($p < 0.05$). HS: high stress profile; IS: intermediate stress profile; LS: low stress profile. * Variables used in the cluster analysis.

h. Bertol et al. (2005) also reported that a 24-h period without food did not generate significant changes in lactate, CK, and glucose levels. However, after 24 h of fasting, glucose concentration tends to increase. In this context, Kephart and Mills (2005) indicated that a 24-h fast results in a 2-kg reduction in feed per pig. Additionally, feeding pigs immediately before transport may be inefficient, as it takes between four and eight hours for the nutrients in the feed to be absorbed in the small intestine, with the majority entering the bloodstream approximately nine hours after ingestion. Consequently, feeding in the final 10 h before transport may not be effective.

The findings suggest that certain physiological markers exhibited greater sensitivity than others within the context of the evaluated scenario. A correlation was observed between cortisol and lactate concentration, as well as between lactate level and glucose. This result is in accordance with the findings of other studies, which indicate that, in situations of stress, animals release cortisol as a catabolic response to mobilise rapid energy. This includes the conversion of glycogen to glucose, which eventually leads to increased lactate and glucose production (Hambrecht et al., 2004; Sommavilla et al., 2017). Furthermore, elevated lactate levels indicate a greater reliance on anaerobic metabolism and increased mobilisation of muscle or liver glycogen stores during the pre-slaughter period, which is attributed to increased physical activity (Edwards et al., 2010). Conversely, a negative correlation was observed between cortisol and glucose. This finding contrasts with

Table 6
Stress coping styles of pigs and their relationships to pre-slaughter logistic variables.

Pre-slaughter logistics operations	Conglomerate 1 HS (n = 92)	Conglomerate 2 IS (n = 142)	Conglomerate 3 LS (n = 124)	p
Total pre-slaughter logistics durations and stress response variables (%)				
Short time (<310 min)	55.4$^{(+)}$	34.5$^{(-)}$	39.2	
Medium time (*>210 min < 300 min*)	19.6	33.1$^{(+)}$	20.0	**
Long time (*>300 min < 420 min*)	25.0$^{(-)}$	32.4	70.8$^{(+)}$	
Transport time and stress response variables (%) ^{B}				
TT1 (<18 min)	44.6 $^{(+)}$	6.1 $^{(-)}$	7.3	
TT2 (60-132 min)	40.2$^{(-)}$	54.2	52.8	***
TT3 (>210 min)	15.2$^{(-)}$	30.3	26.4	
Unloading Time and stress response variables (%) ^{B}				
U1 (<34 min)	69.6 $^{(+)}$	51.4$^{(-)}$	57.6	*
U2 (>40 min)	30.4$^{(-)}$	48.6 $^{(+)}$	42.4	
Lairage Time *and stress response variables (%)* ^{B}				
L1 (<132 min)	66.3	62.0	56.0	ns
L2 (>132 min)	33.7	38.0	44.0	
Sex (%) ^{B}				
Male	55.4	54.9	58.4	ns
Female	44.6	45.1	41.6	
Live weight (%) ^{B}				
<110 Kg	6.5	16.2$^{(+)}$	8.8	
110–130 Kg	30.4$^{(-)}$	50.7$^{(+)}$	44.4	***
>130 Kg	63.0$^{(+)}$	33.1$^{(-)}$	47.2	

Statistical differences according to the Chi-square test, significance differences at $*p < 0.05$ $**p < 0.01$ $***p < 0.001$; CK; Creatine kinase; U; Unloading. $^{(+)}$ and $^{(-)}$ indicate standardized residuals >2 and < −2, respectively. HS: high stress profile; IS: intermediate stress profile; LS: low stress profile.

the results of other studies on pre-slaughter animal management, which indicate a positive relationship between cortisol and glucose (Čobanović et al., 2020; Acevedo-Giraldo et al., 2020).

Glucose levels are considered an indirect indicator of stress. Furthermore, hormones regulate glucose; therefore, glucagon, glucocorticoids, epinephrine, thyroid hormones, growth hormone, and progesterone are hyperglycemic and function by activating gluconeogenesis and glycogenolysis, or by interfering with the glucose that tissues utilize (Mota-Rojas et al., 2011). Prolonged stress in pigs has been demonstrated to increase the release of catecholamines, which can result in the development of cardiovascular disorders such as persistent hypertension and cardiomyopathy (Von Borell and Raoult, 2024). Furthermore, it modifies metabolic processes, predisposing the organism to insulin resistance and the emergence of chronic hyperglycaemia. Consequently, the body's cells are unable to metabolise glucose efficiently, resulting in increased blood glucose levels and decreased cortisol concentrations (Chrousos, 1998). In pigs, a negative relationship between glucose and cortisol could be attributed to various factors such as glycogen depletion, insulin resistance induced by cortisol because of prolonged stress, or complex interactions between the hormones. These factors can lead to a decrease in blood glucose levels, even when cortisol levels are high, thus creating an inverse relationship between both variables. The discrepancies between our findings and those of previous studies may be attributed to the timing of the sampling, which may have occurred during the peak cortisol release, rather than at a point when glucose levels were at their maximum. Additionally, the fatigue status of the pigs may have influenced the results.

The direct relationship between lactate and acute phase proteins (Pig-Map and C-reactive protein) found in our study is consistent with previous studies by Saco et al. (2003) and Piñeiro et al. (2007), who showed increased blood concentrations of these proteins in animals with tissue damage or trauma. Tissue damage produced during pre-slaughter handling activates the inflammatory response, which includes the release of proinflammatory cytokines such as TNF-α and IL-1β (Murtaugh et al., 1996). These cytokines can alter cellular metabolism and increase anaerobic glycolysis, leading to increased lactate production in response to inflammation and tissue damage, which can stimulate an increase in acute phase proteins (Petersen et al., 2004).

4.2. Pre-slaughter logistic chain effects

The findings of this study demonstrate a substantial impact of the total duration of the pre-slaughter logistics chain (comprising pre-transport operations, loading, journey, unloading, lairage, stunning, and bleeding) on physiological stress responses in pigs. The significant increase in cortisol and lactate during the short logistics time (ST) suggests a strong acute stress response, probably linked to initial handling, journey, and novel stimuli associated with the early stages of the process (Mota-Rojas et al., 2012; EFSA, 2022). Conversely, the augmented glucose levels observed in the medium time (MT) group, though not in the long time (LT) group, might signify a transient metabolic peak associated with energy mobilisation as a component of the catabolic stress response. This is followed by potential adaptation or metabolic exhaustion over prolonged periods. Furthermore, elevated creatine kinase (CK) levels in the long logistical time group indicate cumulative muscle damage, likely resulting from physical exertion and prolonged stress during all stages, including transport, unloading, and waiting in the lairage (Brandt et al., 2013; Sommavilla et al., 2017). With regard to C-reactive protein, there does not appear to be a linear relationship between the duration of the logistical process and its concentration in the blood. However, the results of this study indicate that the concentration of LT animals is lower in comparison to that of MT animals. This phenomenon may be attributable to the absence of acute inflammation or partial recovery during the lairage period (García-Celdrán et al., 2012). The absence of significant differences in Pig-MAP levels, as well as the lack of effects of sex or its interaction with logistics time, suggests that these responses are more specific to immediate metabolic and physical stress than to systemic inflammatory processes (Saco et al., 2003) or biological factors such as sex. These findings emphasise the necessity of a comprehensive management strategy for the pre-slaughter logistical process, with the objective of minimising stress and its physiological consequences (Miranda-de la Lama et al., 2014). This should be achieved by prioritising interventions that reduce the physical and metabolic burden accumulated during the pre-slaughter logistic chain.

When analysing transport time as a separate element of the pre-slaughter logistic chain, significant variations in physiological stress indicators are observed, highlighting transport as a pivotal link in the stress response of animals prior to slaughter. In particular, the present study found that short-duration transport (TT1) was associated with higher concentrations of cortisol and lactate, as well as a decrease in glucose and CK concentrations when compared to longer journeys (TT2 and TT3). The findings indicate that a substantial number of stressors, concentrated within a limited timeframe, can compromise the animals' capacity for travel (Pérez et al., 2002; Machado et al., 2022). Conversely, animals transported in TT2 and TT3 exhibited reduced concentrations of cortisol, glucose, and lactate, although CK levels were elevated in TT3, suggesting potential muscle fatigue (Brandt et al., 2013; Sommavilla et al., 2017). The findings of this study demonstrate that intermediate journeys or TT2 would be less detrimental to the animals' physiology in comparison to TT1 and TT3. The absence of pertinent associations in both Pig-Map and CRP indicates that, at least within the parameters of this study, transportation, despite its acute impacts, did not elicit a protracted systemic response of stress or inflammation.

When analysing the effect of transport and unloading time, we found a series of interactions that show the particular effects of these two links in the pre-slaughter logistics chain. Specifically, the group with the shortest transport and unloading times (TT1–U1) exhibited the highest concentrations of cortisol and CRP, along with the lowest glucose values. This suggests a catabolic stress and inflammatory response compared to the other groups (EFSA, 2022). This could be related to more intense and concentrated handling and transport over a shorter period of time, which would have hindered both homeostasis and the initiation of adaptation mechanisms (Kobek-Kjeldager et al., 2023). In contrast, CK increased gradually towards the groups exposed to longer transport, particularly in the group that also experienced longer unloading (TT3-U2), which could reflect progressive muscle deterioration due to both physical stress and fatigue (Brandt et al., 2013; Miranda-de la Lama et al., 2021). Conversely, Pig-Map concentrations exhibited a decline in the TT3–U2 group, indicating that prolonged stress may diminish certain acute phase response mechanisms, consistent with findings from other studies (Piñeiro et al., 2007).

4.3. Coping profiles

The theory of coping styles posits that animals exhibit consistent behavioural and physiological responses to cope with challenges, which can be characterised on a continuum from reactive to proactive (Koolhaas et al., 1999). In accordance with this theory, pigs can be distinguished by their consistency in responding to social or environmental challenges, as well as over time (Janczak et al., 2003). In this sense, the slaughterhouse constitutes a challenging and novel scenario for the animals, and it is to be expected that the physiological responses will vary individually as part of the coping strategies that may be employed (Hedlund and Løvlie, 2015). The results of our study indicate the existence of three profiles of pigs based on their physiological stress response (glucose, lactate and cortisol). These profiles were identified by hierarchical cluster analysis and are characterised as follows: high stress (HS), intermediate stress (IS) and low stress (LS). Furthermore, these profiles were associated with the logistics of pre-slaughter operations. The physiological response and its relationship with pre-slaughter logistics among the different pig profiles demonstrated statistically significant differences. Consequently, stress was effectively managed by some individuals, whereas others exhibited more pronounced physiological changes (Geverink et al., 2003). Some studies have indicated that elevated levels of blood cortisol, lactate and CK are indicative of stress (Terlouw and Rybarczyk, 2008; Sommavilla et al., 2017). Indeed, there is a paucity of research that provides an individual characterisation of the physiological response of pigs during the pre-slaughter period.

However, there are on-farm studies that characterise the individual animal response. For example, Kanitz et al. (2019) identified three groups of pigs (high, medium and low resistance) when exposed to various stressors, including the introduction of new objects in the pen and exposure to an open field. In contrast, Melotti et al. (2011) and Geverink et al. (2003) employed the standardized back test paradigm to categorise pigs into two distinct types: proactive and reactive. Pigs that are classified as proactive exhibit an active response to stressful situations, such as fight or flight, territorial control and aggression. In contrast, pigs that are classified as reactive display less aggressive behaviour, a more cautious demeanour, a tendency to avoid risks and a high level of attention to environmental cues (Bolhuis et al., 2006). From a physiological perspective, our study suggests that animals classified as HS correspond to a reactive coping strategy characterised by high HPA axis activation and pronounced parasympathetic responses. In contrast, IS animals exhibit traits consistent with a proactive style, characterised by greater sympathetic activation and a moderate HPA axis response. Finally, LS animals appear to adopt a passive strategy like the flexibility and adaptability observed in some reactive individuals who survive by actively avoiding danger.

The HS pigs exhibited elevated blood concentrations of lactate, cortisol and CK. Although the glucose concentration was lower than that observed in IS pigs, it remained higher than the basal levels for the species (75–150 mg/dL; Acevedo-Giraldo et al., 2020). A substantial body of evidence indicates that animals with the halothane gene (RYR1) are particularly vulnerable to IS (Nonneman et al., 2012). Nevertheless, there is a paucity of information regarding the characterisation of the stress response in animals that do not carry the halothane gene but are highly susceptible to stress during pre-slaughter handling. Our findings align with those of Aaslyng and Gade (2001), who reported elevated lactate and cortisol levels in high stress finishing pigs subjected to rough handling. Furthermore, Kanitz et al. (2019) observed elevated plasma levels of noradrenaline in pigs categorised as proactive or high-resistance in farrow-to-finish pigs. Furthermore, it is conceivable that HR animals display heightened reactivity in stressful circumstances, thereby elevating their susceptibility to injury and inflammation (Korte et al., 2005). Similarly, Ruis et al. (2002) observed elevated levels of catecholamines and ACTH in HR pigs 24 h after the animals were mixed. In contrast, Rosochacki et al. (2000) observed that when pigs were immobilized for five minutes, plasma cortisol levels increased rapidly, reaching their peak between 10 and 15 min after the onset of immobilization. Consequently, HS pigs are likely to be the most susceptible to stressful situations, such as transport, unloading and short logistics, due to their accelerated physiological response to stress.

It has been demonstrated that IS pigs exhibit a moderate physiological alteration (Reimert et al., 2014). These animals display elevated levels of lactate, glucose and CK, while cortisol levels remain within the normal range (Rey-Salgueiro et al., 2018). Elevated lactate, glucose and CK levels are indicative of extreme physical stress or muscle injury. Consequently, these animals would be expected to rely more heavily on anaerobic metabolism due to tissue hypoxia or intense exercise (Brandt et al., 2015). Our findings align with those of Urrea et al. (2021), who observed elevated lactate and CK levels in pigs subjected to transportation at three distinct densities and with injuries. Furthermore, studies have been conducted on the on-farm animal response, as evidenced by the findings of Kanitz et al. (2019). These studies have reported that pigs with lower cortisol concentrations exhibited intermediate reactions compared to those that were proactive and reactive (58.08 ng/ml, 63.58 ng/ml and 60.87 ng/ml). Furthermore, reactive pigs exhibit elevated levels of immunoglobulin M (IgM), indicative of an adaptive immune response that prepares the organisms to confront immunological challenges in stressful circumstances. Conversely, the logistics and transportation of IS pigs, whether short, medium or long, have no impact. Krause et al. (2017) indicate that low-resistance (LR) pigs are more successful in reversal learning in the Back test. Despite experiencing discomfort and physiological changes, IS pigs were able to adapt and manage these situations relatively normally.

LS pigs are distinguished by cortisol secretion levels that are within the normal range (e.g. <166 μmol/L; Rey-Salgueiro et al., 2018). Nevertheless, a moderate alteration in metabolic balance was observed in comparison to the HS and IS pigs. The LS pigs exhibited lower levels of lactate, glucose and creatine kinase (CK). Nevertheless, these levels remain elevated relative to basal values in pigs. However, the physiological response to stress and immune activation is not uniform across individuals, as it is organised differently depending on their coping profiles and responses to various situations. It is also important to note that the immune organisation and response is not static but rather varies over time due to changes in the environment, particular experiences and other contextual variables (Krause et al., 2017). It can be hypothesised that LS pigs would be the most prepared to face and adapt to stressful situations in the pre-slaughter process due to their prolonged exposure to the logistic chain, which may have a greater capacity to adjust and habituate to the environment and pre-slaughter operations. While this phenomenon requires further study, this initial approach may provide insight into how individual animals respond physiologically to different stressors during pre-slaughter logistic chain.

5. Conclusions

In conclusion, the results obtained show that pigs subjected to short transport times have a more

pronounced stress response compared to those experiencing longer journeys. It is important to note that all animals in the study were subjected to 12 h of fasting and at least 60 min in the loading period at the farm of origin. Another relevant finding is that stress responses show individual variations, which is related to the pigs' coping profiles when faced with the challenges of the pre-slaughter logistics chain. These results emphasise the necessity to balance animal welfare and logistical efficiency, and on this basis, it is recommended to prolong the lairage time of animals subjected to short logistics, allowing for post-transport recovery. However, it is essential to acknowledge certain limitations of this study. First, the research was conducted using a single pig genetic line within a highly mechanized logistics chain, which limits the generalizability of the findings to other production contexts. Additionally, the absence of complementary behavioural assessments means that, although informative, the physiological stress indicators may not fully capture the animals' overall welfare. Future studies should focus on identifying optimal resting times during journeys and short logistics to mitigate their impact on animal stress. In addition, it would be valuable to study behaviours associated with physiological stress profiles and coping profiles, with the aim of identifying animal profiles without the need for blood sampling. These behaviours could be monitored through video recording and artificial intelligence (AI) algorithms. The findings emphasise the necessity to formulate efficacious outreach strategies, such as the utilisation of social media and alternative communication platforms, to counteract misinformation and transparently convey the meat industry's ongoing endeavours in research, technology, and logistics to enhance pig welfare throughout the pre-slaughter logistics chain.

CRediT authorship contribution statement

Nancy F. Huanca-Marca: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Laura X. Estévez-Moreno:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adriana P. Pastrana-Camacho:** Visualization, Writing – proofreading and editing. **Gustavo A. María:** Conceptualization, Validation. **Matilde Piñeiro:** Visualization, Methodology. **Genaro C. Miranda-de la Lama:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this paper.

Acknowledgments

The Spanish Ministry of Industry, Trade and Tourism (Innovative Business Clusters; Project Opticarn, AEI 2022/2054) funded this study. Thanks to the Santander Bank and the University of Zaragoza for the Latin American Scholarship PhD Programme of Nancy F. Huanca-Marca from Peru.

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