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Article

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Posted Date: October 21st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11234272/v1>

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Additional Declarations: No competing interests reported.

Effect of Stocking Density and Crate Height on the Welfare of Fattening Rabbits During Transport to the Slaughterhouse under Mediterranean Autumn Conditions

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Abstract

Recommendations issued by the European Food Safety Authority (**EFSA**) for rabbit transport propose greater space allowance and crate height than those commonly used under European commercial conditions, aiming to allow all rabbits to adopt ventral recumbency simultaneously, change position, and sit upright without their ears contacting the crate ceiling. Nevertheless, empirical evidence demonstrating welfare benefits from these recommendations under commercial transport conditions remains limited. This study aimed to characterize microclimatic conditions and investigate whether stocking density and crate height influence rabbit welfare during commercial transport under Mediterranean autumn conditions. For rabbits within the commercial slaughter weight range in Spain (2.0–2.5 kg), EFSA recommends a stocking density of 47–50 kg/m² and a crate height ≥35 cm. Two commercial transports were evaluated using a 3 × 2 factorial design combining three stocking densities: representing approximately the EFSA recommendation (**LOW**: 50–51 kg/m²), common commercial practice (**MED**: 72–73 kg/m²) and the upper commercial range (**HIGH**: 87–89 kg/m²) and two crate heights: 20 cm (**H20**) representing common commercial practice, and 40 cm (**H40**), compatible with the EFSA recommendation, with 12 replicate crates per treatment combination and transport. The pre-slaughter period, from catching

at the farm to lairage at the slaughterhouse, lasted 5 h 20 min to 6 h and included approximately 85 km of road transport. In-crate temperature, relative humidity, airflow and vibration were characterized across different truck subzones, and rabbit welfare was evaluated through behavioural, thermophysiological and metabolic responses. Despite external conditions of 3–11 °C and 74–98% relative humidity, in-crate conditions were warmer and spatially variable (10–29 °C; relative humidity: 35–90%; transit air speed: ≤ 4.5 m/s; vibration: 1.5–4.9 m/s²). Behaviourally, immobility predominated (68.3 – 99.5%). Upright posture was rare in H40 (pre-transit: 1.7–4.6%; transit: 0.0–0.2%), while unused crate space occurred mainly at LOW and correlated positively with immobility ($r = 0.425$; $p < 0.001$). Rabbits also huddled and avoided areas exposed to incoming airflow. BW loss averaged 75 g (3.1% of initial BW; range: 0–160 g) and rectal temperature decreased by 0.6 °C (range: -2.5 to 0.6 °C), with 12.8% hypothermic after transport and lairage. Neither stocking density nor crate height affected thermophysiological (BW loss, rectal temperature change, hypothermia probability) or metabolic responses (haematocrit, glucose, CK, LDH, NEFAs, corticosterone). Overall, lower stocking density and greater crate height provided no detectable welfare benefit under the transport conditions evaluated.

Keywords: animal, pre-slaughter, space allowance, container, thermal stress.

Implications

Reducing stocking density and providing greater crate height have been recommended to protect rabbit welfare during transport, largely based on planimetric estimates due to the limited empirical evidence available from rabbit transport studies. This study examined the welfare implications of these

recommendations during commercial transport under Mediterranean autumn conditions. Neither reducing stocking density nor increasing crate height did not result in measurable improvements in the welfare indicators assessed. These findings contribute empirical evidence for policymakers, regulators and the rabbit industry when establishing stocking density and crate height requirements for rabbit transport.

Introduction

Approximately 74 million farmed rabbits are transported annually to slaughterhouses for slaughter within the European Union, with Spain accounting for approximately 40% of European Union rabbit production. Rabbits are typically slaughtered at a body weight of 2.0–2.5 kg in Spain, whereas higher slaughter weights are common in other European Union countries (European Rabbit Association, personal communication). Rabbit transport is almost exclusively conducted by road, and approximately 99% of journeys remain within national borders (EFSA AHAW Panel, 2022). In Spain journey durations are generally shorter than 2 h, although the entire transport process extends beyond the road journey and includes catching, crating, loading, transit, unloading and lairage at the slaughterhouse. Rabbits are also commonly fasted for 4–6 h before transport, during which feed and water are withheld (European Rabbit Association, personal communication). Therefore, welfare assessment should consider the entire transport process rather than the transit alone.

Under European commercial conditions, rabbits are generally transported at stocking densities of approximately 60–90 kg/m², with around 70 kg/m² being the most common, in crates typically 20–30 cm high (European Rabbit

Association, personal communication). However, European Union legislation does not currently establish rabbit-specific requirements for space allowance (commonly expressed as stocking density) or crate height during transport. As part of the revision of European Union animal welfare legislation initiated under the Farm to Fork strategy, the European Union requested the European Food Safety Authority (**EFSA**) to prepare updated scientific opinions on the welfare of animals during transport. For rabbits, EFSA proposed minimum recommendations for space allowance and crate height intended to safeguard welfare during transport (EFSA AHAW Panel, 2022).

For rabbits weighting 2.0–2.5 kg, EFSA AHAW Panel (2022) recommended a minimum space allowance of 200–215 cm²/kg, equivalent to a maximum stocking density of approximately 47–50 kg/m². This recommendation was derived from an allometric equation estimating the space required for rabbits to lie simultaneously in ventral recumbency and with the possibility to change posture (Petherick and Phillips, 2009). For crate height, EFSA AHAW Panel (2022) recommended sufficient vertical space for rabbits to sit upright with the ears fully extended without touching the ceiling, resulting in a minimum recommended height of 35 cm for rabbits within this body weight range. This posture was considered relevant to allow heat to be dissipated through the ears under warm conditions. However, EFSA acknowledged the lack of evidence that rabbits naturally adopt this posture during transport, when sufficient vertical space is available.

Thus, both recommendations relied largely from planimetric calculations and theoretical assumptions because empirical evidence linking space allowance or crate height to rabbit welfare outcomes during transport was remarkably

limited. Nevertheless, these recommendations were subsequently incorporated into the European Commission proposal for revised animal transport legislation published in December 2023 (European Commission, 2023a; 2023b).

Experimental validation of these assumptions is particularly relevant in rabbits because their behavioural use of space may differ from that of other livestock species. Rabbits have evolved to spend prolonged resting periods inside underground burrows, where they adopt compact postures and remain in close physical contact with conspecifics, emerging mainly during twilight to forage (Finzi, 1991). Their spatial requirements may therefore not be adequately represented solely by the theoretical floor area required for all individuals to adopt a particular posture simultaneously. Similarly, providing sufficient vertical space to adopt an upright posture does not necessarily demonstrate that rabbits are motivated to use this additional space during transport. Consequently, whether the proposed planimetric estimates translate into measurable welfare benefits under commercial conditions requires empirical validation.

Transport itself exposes animals to multiple and potentially interacting stressors, including feed and water deprivation, handling, thermal challenges, motion and vibration, social interactions, restriction of movement and novelty, whose intensity may vary across the different phases of the transport process (EFSA AHAW Panel, 2022). Animals respond to such challenges through behavioural and physiological coping mechanisms (Broom, 1986; 1991) and may require the mobilisation of biological resources. As long as the animal successfully copes with the challenge, this biological cost may be reflected in measurable changes such as body weight loss, alterations in body temperature, dehydration, mobilisation of energy reserves, tissue damage or activation of

neuroendocrine stress responses. However, when the adaptive capacity of the animal is exceeded, physiological failure may ultimately result in death. Consequently, welfare during transport should not be evaluated solely from mortality or clinical observations, but also through a combination of behavioural, thermophysiological and metabolic indicators capable of detecting the biological cost associated with coping with transport-related stressors.

A series of complementary studies was undertaken to experimentally evaluate the assumptions underlying the proposed recommendations. Rabbit postural requirements were first examined under controlled climatic conditions (Contreras-Jodar et al., 2025a), followed by an evaluation of the combined effects of stocking density, crate height and thermal environment also under controlled climatic conditions (Contreras-Jodar et al., 2025b). These factors were subsequently assessed under commercial transport conditions during mild Mediterranean summer conditions (Contreras-Jodar et al., 2026). However, the thermal environment experience during commercial transport varies seasonally, and the implications of stocking density and crate height may differ under cooler conditions. In particular, stocking density may affect not only the space available for movement and postural adjustment but also heat exchange among grouped rabbits, while increased height may alter exposure to airflow. Evaluating the same factors under autumn conditions therefore provides a complementary thermal scenario in which the assumptions underlying the proposed recommendations can be assessed.

Accordingly, the present study aimed to characterize the in-crate conditions within a commercial rabbit transport truck and to evaluate the effects of three target stocking densities (50, 70, and 90 kg/m²) and two crate heights

(20 and 40 cm) on rabbit welfare during to slaughter under Mediterranean autumn conditions. Welfare was assessed using behavioural, thermophysiological and metabolic indicators.

Material and methods

The experimental procedures and analytical approaches used in the present study were based on those previously described by Contreras-Jodar et al. (2026). Methodological details are provided below where necessary to describe the specific experimental design and conditions of the present study.

Animals, transport and treatments

Two batches (A and B) of mixed-sex fattening rabbits were transported from a commercial farm to a slaughterhouse on two different days in November 2025 in Spain. The rabbits were commercially reared animals owned by the integrating company and destined for slaughter for human consumption. Permission to conduct the study was obtained from the integrator company. The experimental protocol was approved by the Ethics Committee for Animal and Human Experimentation (CEEAH) of the Universitat Autònoma de Barcelona (UAB) under protocol CEEAH 5682-CEEA-UAB on 23 April 2024. The study was conducted in accordance with the local legislation and institutional requirements.

Batch A (n = 2532 rabbits of Hypharm genetics; mean of 2.46 kg BW/rabbit) and batch B (n = 2532 rabbits of Hypharm genetics; mean of 2.42 kg BW/rabbit) were transported on separate days using the same truck configuration, equipped with 16 plastic transport trolleys (2 wide × 8 long × 2 levels in height). Each trolley contained 8 or 10 crates arranged in two vertical

stacks of 4 or 5 crates per side (left and right; see Figure 1), forming a single trolley unit. In total, the truck accommodated 288 crates. Crates measured 111 cm length × 70 cm width × 20 cm height.

Although both batches were transported under commercial conditions, specific adaptations were introduced to assess the effects of stocking density and internal crate height during transport. Three target stocking densities (50, 70 and 90 kg/m²) were combined with two internal crate heights (20 cm and 40 cm), resulting in six experimental crate treatments defined a priori. The 20-cm height (**H20**) corresponded to the standard commercial crate, whereas the 40-cm height (**H40**) was obtained by removing the ceiling separating two vertical stacked crates.

The number of rabbits allocated to each crate was determined according to the mean BW of each batch to approximate the target stocking densities. In both batches, crates assigned to 50, 70 and 90 kg/m²) targets contained 16, 23 and 28 rabbits, respectively. As the resulting stocking densities differed slightly from the nominal targets, treatments were subsequently described according to the densities actually achieved across batches: 50–51 kg/m² (**LOW**), 72–73 kg/m² (**MED**), and 87–89 kg/m² (**HIGH**). Their combination with the two crate heights resulted in six experimental crate treatments: LOW-H20, LOW-H40, MED-H20, MED-H40, HIGH-H20 and HIGH-H40.

Twelve spatial replicates of each crate treatment were systematically distributed throughout the truck to account for potential spatial variation. For this purpose, the truck was divided into 12 subzones defined by the combination of three longitudinal positions (front, middle, and rear), two vertical positions (upper and lower), and two lateral positions (left and right).

The transport process was divided into three main phases: pre-transit (catching, crating, and loading onto the truck), transit (truck journey from farm to slaughterhouse) and post-transit (unloading and lairage at the slaughterhouse). The duration of each phase for each batch is presented in Figure 2. The overall process, from the start catching to the end of lairage, lasted 5 h 20 min in batch A and 6 h in batch B and included approximately 85 km of road transport. The longer duration in batch B resulted from an operational delay before loading, caused by a mechanical failure of the forklift that required the transporter to return to the company facilities for repair.

As direct measurements of external environmental conditions were not available during the whole transport phases, ambient temperature and relative humidity (**RH**) were retrieved from the closest meteorological station to the transport route and plotted (Figure 3).

Characterization of transport conditions

Environmental conditions during transport were monitored using temperature and humidity sensors, anemometers, a sound level meter and accelerometers. Temperature was recorded at 1-min intervals using 52 sensors (iButton ThermoChron DS1922L Temperature Loggers, iButtonLink, LLC, Wisconsin, USA), relative humidity was recorded also at 1-min intervals using 12 sensors (HOBO MX2301 Temp/RH Data Logger, HOBO Data Loggers, Bourne, Massachusetts, USA), while air speed was monitored using five anemometers (Kestrel 5000 Environmental Meter, Nielsen-Kellerman Company, Pennsylvania, USA). Crate vibration during transport were recorded using 12 accelerometers (HOBO Pendant G Data Logger, HOBO Data Loggers, Bourne, Massachusetts,

USA). Sensors were distributed across the different truck subzones and positioned in the transport crates, at approximately rabbit head level. Noise levels (or intensity) were measured just before departure within the engine vehicle running using a sound level meter (Testo 815, Instrumentos Testo, S.A., Cabrils, ES).

Behaviour

Rabbit behaviour during transport was continuously recorded using six video cameras (NfMaxone P, NFMaxOne, ES), with one camera assigned to each crate treatment. Recordings were analysed during three periods: the hour preceding departure, transit, and the first hour after arrival at the slaughterhouse. Behavioural observations were performed using BORIS software v.7.13.8 (Friard and Gamba, 2016) at 5-min intervals according to the ethogram presented in Table 1. At each observation, the proportion of rabbits displaying each posture and behaviour was calculated relative to the total number of rabbits in the corresponding crate. Unused space was also assessed at each by estimating the number of additional rabbits that could fit within the unoccupied floor area.

Thermophysiological response

A subset of rabbits was randomly selected at the farm of origin and individually marked at the farm on the day before transport (batch A: 144 rabbits; batch B: 144 rabbits). Two marked rabbits were assigned to each replicate crate, with 12 replicates per treatment. Following a 6 h feed and water withdrawal period, BW and rectal temperature (**RT**) were recorded before transport and again upon arrival at the slaughterhouse. At arrival, marked rabbits were also

assessed for urine-soiled fur and lethargy using the criteria described by Contreras-Jodar et al. (2026). Dead-on-arrival (**DOA**) rabbits were recorded both within the experimental subset according to crate treatment and for the entire batch.

Metabolic response

Blood samples were collected by ear vein puncture prior to stunning and slaughter to assess haematocrit, glucose, lactate dehydrogenase (**LDH**), creatine kinase (**CK**), non-esterified fatty acids (**NEFAs**), and corticosterone (**CORTI**). Samples from both batches were processed, stored and analysed following the procedures described by Contreras-Jodar et al. (2026), without any modifications.

Following completion of the measurements, rabbits underwent routine commercial slaughter for human consumption according to the standard procedures of the slaughterhouse. Rabbits were stunned using head-only electrical stunning in accordance with European Union legislation on the protection of animals at the time of killing (Council Regulation (EC) No 1099/2009) and subsequently killed by exsanguination through neck cutting.

Statistical analysis

All data processing, statistical analyses, and graphical representations were performed using R software version 4.5.1. Linear mixed models were fitted using the “nlme” package (Pinheiro and Bates, 2024) and marginal (R^2_m) and conditional (R^2_c) coefficients of determination were obtained using the “MuMIn”

package (Barton, 2025). Statistical significance was set at $p < 0.05$ and a tendency when $p < 0.10$.

Characterization of transport conditions

Temperature, RH and air speed were summarised at each recording time by averaging measurements from sensors located within the same truck subzone and plotted over actual clock time. Crate vibration was derived from tri-axial acceleration measurements and expressed as root-mean-square acceleration over 1-min intervals. The three axis-specific root mean square values were subsequently integrated into an overall vibration measure following Morris et al. (2021) and ISO 2631-1 guidance.

Noise measurement was characterised using A- and C-weighting sound pressure levels (dB(A) and dB(C), respectively). The difference between both measurements ($\Delta\text{dB} = \text{dB(C)} - \text{dB(A)}$) was used to describe the contribution of low-frequency sound. Measurements were obtained in predefined truck subzones while the vehicle was stationary with the engine running. Given the limited number of measurements available per subzone and batch, noise variables were only summarised descriptively.

Behaviour

For each observation, the proportion of rabbits displaying each behaviour was calculated relative to the number of rabbits present in the corresponding crate and summarised according to crate treatment and transport phase. Behavioural assessment was performed according to the ethogram presented in Table 1. Observer reliability was supported by the inter-observer agreement

conducted in Contreras-Jodar et al. (2026), in which the observer involved in the present study achieved a Cohens' kappa coefficient of 0.851, indicating excellent agreement (Fleiss et al., 2003). Behavioural outcomes were analysed using generalized linear mixed models with a binomial distribution and logit link function were fitted to assess the effects of stocking density, crate height and observation phase as fixed effects. Stocking density (LOW, MED and HIGH), crate height (H20 and H40), transport phase (pre-transit, transit and post-transit), and their interactions were included as fixed effects, while the experimental unit was included as a random effect to account for repeated observations from the same crate treatment within batch. For behavioural variables characterized by an excess of zero observations, zero-inflated binomial models were fitted when this resulted in an improved model fit. When significant effects or interactions were detected, estimated marginal means were obtained on the response scale and pairwise comparisons were performed, with Tukey adjustment when applicable.

Unused crate space was classified according to the estimated number of additional rabbits that could occupy the unoccupied floor area: none (0 rabbits), limited (1–2 rabbits), moderate (3–4 rabbits), or substantial (5–6 rabbits). This variable was summarised descriptively as the percentage of observations within each category.

Video sequences illustrating rabbit behaviour during transit in LOW-H20 and HIGH-H40 crates are provided in supplementary material.

Thermophysiological response

For each marked rabbit, BW loss and change in RT were calculated by subtracting the final value recorded at lairage from the corresponding value measured after 6h fasting period.

Individual BW loss and change in RT values were averaged within each crate, which was subsequently considered the experimental unit. Then, they were analysed using a linear mixed model including stocking density (LOW, MED, HIGH), crate height (H20, H40) and their interaction as fixed effects. Crate position within the truck, nested within batch (A and B), was included as a random effect to account for spatial variability.

The potential contribution of crate location within the truck to BW loss and change in RT was further explored using additional linear mixed models. Longitudinal (front, middle, rear), vertical (upper, lower), and side (left, right) positions, together with their two- and three- way interactions among these factors, were included as fixed effects, with batch included as a random effect. When a significant effect was detected estimated marginal means were compared using Tukey adjustments.

Thermal state was defined by classifying both initial and final RT into three categories according to Kahn and Line (2010): hypothermia ($< 38.6^{\circ}\text{C}$), normothermia ($\text{RT } 38.6 - 40.1^{\circ}\text{C}$), or hyperthermia ($\text{RT } > 40.1^{\circ}\text{C}$). The effects of stocking density, crate height, and their interaction on the probability of hypothermia after transport and lairage were evaluated using a binomial logistic regression, with rabbits classified as hypothermic or non-hypothermic. The probability of a rabbit being wet after transport and lairage was similarly analysed using binomial logistic regression, including stocking density, crate height, and their interaction as fixed effects. Pairwise comparisons among crate treatments

were performed using estimated marginal means, with Tukey adjustment for multiple comparisons. The association between wetness and the probability of hypothermia was also evaluated using binomial logistic regression.

Lethargy and DOA were analysed only descriptively due to the absence of lethargic rabbits and the lack of variation in the number of DOA between batches, respectively.

Metabolic response

For biochemical variables (haematocrit, glucose, CK, LDH, NEFAs, and CORTI), individual measurements were averaged within each crate, which was considered the experimental unit. Data were assessed for normality and homoscedasticity prior to analysis, and variables not meeting the normality assumption were log-transformed. Crate-level means were then analysed using linear mixed models, with stocking density (LOW, MED, HIGH), crate height (H20, H40) and their interaction included as fixed effects. Subzone within the truck, nested within batch (A and B), was included as a random effect to account for spatial variability.

For metabolic variables in which the random component made a relevant contribution to the variance explained by the model, as indicated by a substantially higher R^2_c than R^2_m , the potential contribution of spatial variability within the truck was further explored using the same approach described for BW loss and change in RT.

Results

Characterization of transport conditions

Temporal changes in in-crate temperature, RH and air speed throughout the different transport phases for both batches are presented in Figure 4.

In batch A, in-crate temperature showed clear temporal and spatial variation throughout transport. During the catching, mean temperatures across subzones ranged from approximately 14 to 24 °C and generally increased over time, although the magnitude of this increase differed among truck subzones. During loading, temperatures remained within a similar range, approximately 17 to 25 °C, with the highest values recorded in the upper front and middle subzones. Following truck departure, temperatures decreased across all subzones and remained generally between 10 to 20 °C during transit, with higher temperatures recorded in the middle-lower-right subzone and lower temperatures in the middle-upper-left and rear-lower-right subzones. Following unloading, temperature initially increased in all truck locations, reaching approximately 15 to 29 °C, but subsequently decreased markedly during lairage. This decline coincided with the progressive removal of the sensors from the truck as rabbits were taken for slaughter and therefore does not represent changes in the thermal conditions experienced by rabbits remaining inside the truck. RH showed greater spatial variation than temperature throughout transport. During catching and loading, values were generally between 60 and 90%. During transit, RH ranged approximately from 50 to 85%, with variation among subzones but no consistent spatial pattern. During unloading and lairage, a clearer spatial differentiation appeared, with RH generally remaining around 50–90% in the front, while values in the middle subzones ranged approximately between 40 and 75%. No RH data were available for rear section of the truck. Air speed was negligible during catching, loading, and unloading and lairage. During transit, air speed increased

markedly but showed spatial variation, with the highest values recorded in the front subzone, reaching peaks above 4 m/s. Values in the middle subzones were generally lower, mostly remaining below 2 m/s, whereas air speed was negligible in the rear subzone. Vibration exposure during catching ranged from 0.20 to 0.63 m/s^2 across subzones. Values increased during loading (0.42 to 1.22 m/s^2), while during transit, the highest vibration was recorded in the upper-front of the truck, reaching 4.13 m/s^2 , whereas values in the remaining subzones showed values between 1.47 to 2.19 m/s^2 . During unloading and lairage values ranged from 9.08 to 9.91 m/s^2 . However, these values coincided with the progressive removal and handling of the accelerometers and therefore may not represent the vibration experienced by rabbits during lairage. Regarding noise exposure, mean dB(A) was 67.9 dB (range: 61.6–72.8 dB(A)). Mean dB(C) averaged 79.2 dB (range: 74.8–82.6 dB(C)). The highest noise levels were recorded in the front area of the truck, whereas the lowest values were observed in the rear area. ΔdB averaged 11.3 dB (range: 9.8–13.2 dB).

In batch B, in-crate temperature ranged approximately between 12 to 29 °C across subzones, with the highest temperatures recorded in the front section of the truck. During loading, temperatures generally ranged from approximately 14 to 27 °C, with spatial differences among subzones but no consistent pattern according to vertical position or side. Following truck departure, temperatures decreased across most subzones and remained generally between 10 and 25 °C during transit. Temperatures were generally higher in the front subzones. Following unloading, temperatures increased in most subzones, reaching approximately 15 to 27 °C. As lairage progressed, temperatures subsequently decreased in several subzones, coinciding with the progressive removal of the

sensors from the truck as rabbits were taken for slaughter. RH during catching ranged from 40 to 80%, during loading from 35 to 75% and during transit from 40 to 75% with no consistent spatial pattern. During unloading and lairage, spatial variability increased, with RH ranging from 30 to 80%. Air speed was negligible during catching, loading, and unloading and lairage. During transit, air speed exhibited intermittent peaks of up to 4.5 m/s in front-bottom, 0.9 m/s in middle-bottom while remaining close to zero in the other subzones. Vibration exposure during catching ranged from 0.28 to 0.62 m/s² across subzones. Values increased during loading (0.37 to 1.35 m/s²), while during transit, the highest vibration was recorded in the upper-front of the truck reaching 4.90 m/s², whereas values in the remaining subzones ranged from 1.47 to 2.68 m/s². During unloading and lairage values reached up to 9.95 m/s². The elevated values recorded during this phase occurred while accelerometers were being progressively removed and handled and may therefore not reflect the actual vibration exposure during lairage.

Regarding noise exposure, mean dB(A) was 66.0 dB (range: 62.2–70.2 dB(A)). Mean dB(C) was 81.1 dB (range: 78.3–85.5 dB(C)). The highest noise levels were recorded in the front area of the truck, whereas the lowest values were observed in the rear area. Δ dB averaged 15.1 dB (range: 12.7–16.5 dB).

Behaviour

The proportion of behaviours displayed recorded across transport phases, stocking densities and crate heights is presented in Table 2. Example video clips illustrating rabbit behaviour during transit are provided in the supplementary material and can be accessed via the accompanying QR codes.

Rabbit behaviour was strongly dominated by immobility, which increased markedly once the vehicle was in motion. Immobility ranged from 55.6 to 76.7% during pre-transit and significantly increased to 84.9–95.6% during transit in all crate treatments ($p < 0.001$). During post-transit, immobility remained high (74.1–90.0%) and decreased relative to transit.

Other behaviours and postures were generally infrequent. Sitting or standing was observed only during pre-transit and exclusively in H40 crates, accounting for 1.8–2.3% of observations across stocking densities, and was not observed during transit or post-transit. Standing up was similarly infrequent, occurring mainly during pre-transit in H40 crates (1.8 – 3.3%) and being virtually absent once the vehicle was in motion and not observed during post-transit. Stacking was generally infrequent, although it was more frequently observed in HIGH-H40, accounting for 11.8% of observations during pre-transit, 6.3% during transit and 5.1% during post-transit. Movement was also infrequent ($\leq 3.7\%$ during pre-transit and $\leq 2.3\%$ during transit) and was rarely observed during post-transit. The expression of other behaviours differed among transport phases ($p < 0.001$), whereas no effects of stocking density ($p > 0.693$) or crate height were detected ($p > 0.875$).

Unused crate space was observed across stocking densities and transport phases. During pre-transit, unused space was observed in 39.0% of observations at LOW and 15.8% at MED, whereas no unused space was observed at HIGH. During transit, larger amounts of unused space were observed, particularly at LOW, where 40.0% and 18.0% of observations were classified as moderate and substantial, respectively. At MED, unused space was predominantly limited (57.7%) or moderate (26.9%), whereas at HIGH, 60.0% of observations showed

no unused space and, when present, it was almost exclusively limited (38.0%). During post-transit, unused space at LOW obwas predominantly limited (52.4%), although moderate (4.8%) and substantial (14.3%) unused space was also observed. At MED, 45.8% and 29.2% of observations were classified as limited and moderate, respectively, with no substantial unused space observed. In contrast, 93.8% of observations at HIGH showed no unused, with the remaining 6.3% classified as moderate.

Thermophysiological response

BW, RT, thermal state, and lethargy status after the 6 h of fasting and immediately before crating are presented in Table 3. BW (2.44 ± 0.11 kg; $p = 0.841$) and RT (39.7 ± 0.3 °C; $p = 0.400$) did not differ significantly among crate treatments. Regarding thermal state, normothermia was observed in 91.7 to 100% across crate treatments. Hyperthermia was noted in 0–8.3% of rabbits, while no cases of hypothermia or lethargy were recorded before transport.

After transport, mean BW loss was 75 g (range: 0–160 g). Neither stocking density ($p = 0.335$) nor crate height ($p = 0.999$) had a significant effect on the BW loss, although a tendency for an interaction between both factors was observed ($p = 0.096$). The model yielded $R^2m = 0.048$ and $R^2c = 0.243$. The potential contribution of crate location within the truck was also explored, but neither longitudinal, vertical nor side position, nor any of their interactions, significantly affected BW loss (all $p > 0.05$; $R^2m = 0.098$, $R^2c = 0.311$).

Mean changes in RT was -0.6 °C (range: -2.5–0.6 °C; Figure 5A). Stocking density did not significantly affect the change in RT ($p = 0.180$), whereas a tendency for an effect of crate height was observed ($p = 0.077$). No interaction

between stocking density and crate height was detected ($p = 0.586$). The model resulted in $R^2_m = 0.062$ and $R^2_c = 0.127$. The potential contribution of crate location within the truck was also explored and yielded $R^2_m = 0.351$ and $R^2_c = 0.443$. Change in RT was affected by longitudinal position ($p < 0.001$), whereas no effects of vertical ($p = 0.136$) or side position ($p = 0.415$), or any of their interactions (all $p > 0.05$), were detected. RT decreased more at the front (-0.9 °C) than at the middle (-0.6 °C) or rear (-0.3 °C) positions (both $p < 0.001$), and more at the middle than at the rear ($p < 0.001$). BW loss was not associated with the change in RT ($p = 0.354$; Figure 5B).

The prevalence of the different thermal states, wet rabbits, and lethargy after transport and lairage is presented in Table 4. Regarding thermal state, most rabbits were normothermic, with proportions ranging from 79.2 to 93.8% across treatments. Hypothermia was recorded in all treatments, ranging from 6.2% in HIGH-H40 to 20.8% in MED-H20. However, the probability of hypothermia was not significantly affected by stocking density ($p = 0.171$), crate height ($p = 0.334$), or their interaction ($p = 0.413$). Hyperthermia was limited to a single rabbit in the LOW-H40 treatment (2.1%). The prevalence of wet rabbits differed between MED-H40 and HIGH-H40 (35.4% vs 8.3%; $p < 0.034$), whereas no significant differences were detected among the remaining treatments ($p < 0.05$). Wetness was not significantly associated with the probability of hypothermia ($p = 0.113$). Despite some rabbits had RT compatible with hypothermia, no lethargic rabbits were observed in any crate treatment. No DOA were recorded among the subset of rabbits assessed in either batch A and B rate was 0.00% in the subset of rabbits assessed both in batch A and B, whereas at the whole-truck level, DOA prevalence was 0.04% in both batches.

528

529 ***Metabolic response***

530 The results of the biochemical analyses in blood samples are summarized
531 in Table 6.

532 None of the biochemical variables measured in blood samples was
533 significantly affected by stocking density, crate height or their interaction ($p >$
534 0.05). The mean haematocrit value was $38.1 \pm 2.2\%$ (range: 33.0–45%; median:
535 38.0%), mean glucose was 159 ± 22 mg/dL (range: 115–256 mg/dL; median: 157
536 mg/dL), mean LDH was 277 ± 228 U/L (range: 52–1738 U/L; median: 212 U/L),
537 mean CK was 1612 ± 1178 U/L (range: 364–10.714 U/L; median: 1321 U/L),
538 mean NEFAs was 0.570 ± 0.184 mmol/L (range: 0.180–1370 mmol/L; median:
539 0.550 mmol/L) and mean CORTI was 21.5 ± 7.2 ng/mL (range: 7.7–81.8 ng/mL;
540 median: 21.0 ng/mL). R^2_m values were low across all models (R^2_m : 0.011 –
541 0.066), whereas R^2_c values ranged from 0.066 to 0.453. The largest differences
542 between R^2_m and R^2_c , were observed for glucose, NEFAs and CORTI, for which
543 the potential contribution of spatial variability within the truck was further explored.
544 The subsequent analysis revealed that glucose concentrations were affected by
545 longitudinal position ($p > 0.001$), whereas no effects of vertical ($p = 0.785$) or side
546 position ($p = 0.194$) were detected. Thus, glucose concentration was higher at
547 the front of the truck (176 mg/dL) than at the middle (155 mg/dL) or rear (150
548 mg/dL) positions (both $p < 0.001$), with no difference between the middle and rear
549 positions ($p = 0.144$). NEFA concentrations were affected by vertical position (P
550 $= 0.008$), but not by longitudinal ($p = 0.200$) or side position ($p = 0.588$), with
551 higher concentrations in the lower than in the upper positions (0.597 vs 0.541
552 mmol/L). For CORTI, a tendency for an effect of longitudinal position was

observed ($p = 0.077$), whereas vertical and side positions had no significant effect ($p = 0.730$ and $p = 0.458$, respectively). CORTI concentrations tend to be higher at the front than at the rear of the truck (23.2 vs 19.9 ng/mL; $p = 0.062$), while the remaining comparisons were not significant.

Discussion

Characterization of transport conditions

The two transports were conducted in Spain in November 2025 under relatively cool, but different, external ambient conditions. In batch A, external temperature increased from approximately 6 °C at 09:00 to 10–11 °C by 15:00, whereas batch B started at approximately 3 °C and reached 7–8 °C over the same period. Despite this low ambient temperatures, substantially warmer conditions were recorded inside the transport crates throughout the process, revealing a clear decoupling between ambient and in-crate temperature. Differences exceeding 15 °C between external and in-crate temperatures were observed at particular times and truck positions, with in-crate temperatures approximately 25 °C in batch A and 28–29 °C in batch B. This indicates that ambient temperatures alone provide a limited representation of the thermal conditions actually experienced by rabbits during commercial transport.

This decoupling between ambient and in-crate temperature is consistent with that observed under mild Mediterranean summer conditions by Contreras-Jodar et al. (2026). In that study, ambient temperatures ranged from approximately 26 to 23 °C, whereas in-crate temperatures reached short-term peaks of 30–33 °C during catching and loading period. The present study extends these observations to substantially colder conditions, showing that a considerably

warmer in-crate microclimate can develop even when external temperatures remain below approximately 11 °C. This suggests that the divergence between ambient and in-crate temperature is not restricted to warm-weather transports.

This divergence was already apparent during catching and loading, when in-crate temperature generally increased as rabbits were progressively placed into the crates and occupied crates were transferred to the truck, despite external temperatures remaining comparatively low. The accumulation of metabolic heat produced by the rabbits, together with limited heat dissipation while crates were stationary and the progressive stacking of occupied crates within the vehicle, may have contributed to the development of this warmer in-crate microclimate. The magnitude of the increase, however, differed considerably among crate positions, suggesting that the thermal environment was also influenced by the spatial configuration of the load.

Vehicle movement altered this microclimate but did not eliminate the divergence from external ambient conditions. In-crate temperatures generally declined after departure, likely reflecting increased convective heat loss associated with greater airflow through the moving vehicle, but remained above external temperature and substantial heterogeneity persisted throughout transit. Moreover, the spatial pattern was not consistent between transports. In batch A, the highest transit temperatures were generally observed in the middle-lower section of the truck, whereas in batch B the front showed the highest temperatures during part of the journey. Thus, while external temperature increased progressively in both batches, in-crate temperature followed a different temporal trajectory and its spatial distribution varied between journeys. Together, these findings suggest that the in-crate thermal conditions result from the

interaction between the external environment, animal-generated heat, ventilation during vehicle movement, and crate position, rather than being directly determined by ambient temperature alone.

During unloading and lairage, in-crate temperature profiles diverged further as crates were progressively removed from the truck. At this stage, however, the original truck subzone no longer represented the actual location or environmental exposure of each crate. Consequently, differences observed after unloading cannot be interpreted as spatial gradients within the loaded vehicle and more likely reflect the transition from the truck microclimate to the conditions prevailing in the lairage area.

RH also showed marked temporal and spatial variation within the transport crates, although its distribution was less consistent than that observed for temperature. During catching and loading, in-crate RH varied considerably among crate positions, likely reflecting the progressive introduction of rabbits and the accumulation of moisture from respiration and excreta within an initially poorly ventilated environment. Once the vehicle was in motion, changes in airflow may have facilitated moisture removal, although differences among crate positions persisted and no consistent spatial pattern was reproduced across both transports. This suggests that, as for temperature, the humidity experienced by rabbits within the crates cannot be inferred solely from external ambient conditions, but results from the balance between moisture generated within occupied crates and its removal through ventilation.

A similar phase-dependent pattern was observed under summer conditions by Contreras-Jodar et al. (2026), where greater variation in RH among crate positions occurred during catching and loading, followed by more

homogeneous conditions once the vehicle was in transit. The recurrence of this pattern under contrasting seasonal conditions further supports the importance of vehicle movement and ventilation in determining not only heat but also moisture exchange within the loaded truck. The air-velocity measurements support this interpretation, as air movement within the crates remained minimal while the vehicle was stationary and increased markedly, although irregularly, once transit began.

Vibration exposure was strongly dependent on transport phase, with dynamic acceleration remaining low while the vehicle was stationary and increasing markedly once transit began. During vehicle movement, however, vibration was not uniformly distributed throughout the load, with differences in root-mean-square acceleration among truck subzones. This spatial heterogeneity indicates that rabbits transported within the same vehicle may nevertheless be exposed to different mechanical environments. Such differences may arise from the interaction between vehicle dynamics, road irregularities, suspension characteristics, and the location of the crates relative to the vehicle axis among others. Although root-mean-square acceleration is widely used to quantify vibration magnitude, species-specific exposure limits linking particular vibration levels to adverse welfare outcomes have not been established for rabbits.

Noise exposure also showed a clear spatial pattern within the vehicle, with the highest sound levels recorded at the front and the lowest towards the rear. This longitudinal pattern is consistent with that observed during summer transports (Contreras-Jodar et al., 2026), suggesting that crate location may consistently influence the acoustic environment experienced by rabbits during transit. The greater noise exposure at the front may be related to its proximity to

the engine. However, the biological relevance of the sound levels recorded remains difficult to establish, as species-specific thresholds linking noise exposure during transport to adverse welfare outcomes have not been established for rabbits.

Taken together, the present findings reinforce the importance of characterising transport conditions at crate level rather than relying exclusively on external environmental measurements.

The marked spatial and temporal heterogeneity observed across thermal conditions, air speed, vibration, and noise also indicated that rabbit transported simultaneously within the same vehicle may experience different combinations of environmental and mechanical stressors. However, the welfare relevance of these differences cannot be inferred directly from the magnitude of the physical measurements, particularly for vibration and noise, for which rabbit-specific exposure thresholds remain unknown. Rather, this detailed characterisation of the transport environment provides the basis for interpreting the behavioural, thermophysiological, and metabolic responses observed in the present study and for examining whether differences in exposure within the vehicle translate into measurable differences in rabbit response.

Behaviour

Rabbit behaviour was characterised by a high proportion of immobility across all transport phases, irrespective of the stocking density and crate height. Immobility was particularly high during transit, reaching 84.9–95.6% of observations, compared with 55.6–76.7% during pre-transit, and remained relatively high during post-transit. The predominance of immobility during transit

is consistent with our previous studies under controlled thermal conditions, in which rabbits also spent most of the observation period inactive despite the absence of vehicle motion (Contreras-Jodar et al., 2025a, 2025b), as well as with our previous commercial transport study conducted under summer conditions (Contreras-Jodar et al., 2026). Taken together, these findings suggest that the high levels of immobility observed during transit should not necessarily be interpreted as a behavioural response to vehicle motion itself, but rather as a consistent behavioural pattern of rabbits when confined under transport-related conditions. The somewhat greater behavioural activity observed during the pre- and post- transit phases may, at least partly, have resulted from the handling and movement of the crates during loading and unloading operations, including their displacement by forklift between the farm or lairage area and the truck. Nevertheless, behaviours involving movement and postural changes remained generally infrequent throughout the transport process, with no clear effects of stocking density or crate height.

The limited behavioural effect of crate height was particularly evident in the use of the additional vertical space provided by H40 crates. Sitting or standing with erect ears or not was observed exclusively in H40 crates, confirming that the additional height allowed rabbits to adopt these postures. However, their occurrence was low during pre-transit (1.8–2.3%) and they were not observed during transit and post-transit. Moreover, direct observation of the video recordings showed that rabbits in H40 crates generally remained crouched, maintaining a low body posture rather than occupying the additional vertical space available.

Importantly, the limited use of vertical space does not appear to be specific to vehicle motion. In our previous study conducted in a controlled climatic chamber, rabbits were observed for 8h under markedly different thermal conditions, ranging from 15.3 °C and 63.0% RH to 31.1 °C and 35.5% RH, in the absence of transport-related motion (Contreras-Jodar et al., 2025a). Despite these contrasting thermal conditions, rabbits spent only 0.7–1.2% of the observation time in an upright posture, with no differences among thermal treatments. Notably, this occurred despite rabbits markedly modifying other postures in response to increasing ambient temperature, shifting from lying towards sternal and lateral sprawling. Thus, while rabbits clearly adjusted their resting posture in response to the thermal environment, the use of upright posture remained consistently around 1%, irrespective of thermal conditions. This limited use of vertical space is consistent with observations by Finzi et al. (2005), who studied adult rabbits housed in an experimental cage without vertical restriction and concluded that rabbits did not generally rise above their normal height in a quadrupedal stance, except occasionally during initial exploration. Furthermore, in our previous commercial summer study (Contreras-Jodar et al., 2025c), rabbits similarly made little use of the additional vertical space provided by H40 crates, with sitting upright rarely observed during transit (0.0–0.8%).

The consistently limited use of vertical space may also be considered in the context of the behavioural ecology of the rabbit. As a burrowing species, wild rabbits spend prolonged periods resting underground, where space is naturally constrained and rabbits remain in compact postures and in close physical contact with conspecifics (Finzi, 1991). From this perspective, the limited tendency of

rabbits to use additional vertical space when it is available may not be unexpected.

These consistent observations are particularly relevant in relation to the EFSA AHAW Panel (2022) recommendation that transport crates should provide sufficient height for rabbits to sit in a natural position with their ears extended without touching the ceiling. This recommendation was intended to avoid movement restriction and to allow rabbits to extend their ears for thermoregulation. However, EFSA also acknowledged the uncertainty as to whether rabbits would actually adopt such an upright posture during transport when sufficient vertical space was available. The present findings, together with our previous observations, provide empirical evidence addressing this uncertainty. Although H40 crates allowed rabbits to adopt upright postures, these were rarely expressed. Importantly, our previous climatic chamber study showed that this remained the case even under conditions in which rabbits exhibited clear behavioural thermoregulation through marked changes in lying and sprawling postures (Contreras-Jodar et al., 2025a).

A limited behavioural response to increased vertical space has also been reported in other species during transport. In broiler chickens, Vinco et al. (2016) reported that even when crates provided sufficient vertical space for birds to stand, only around 4% attempted to do so while the vehicle was moving. Moreover, greater vertical space has been associated with balance loss and increased injuries in broiler chickens and turkeys (Wichman et al., 2012; Vinco et al., 2016; Di Martino et al., 2017), while increasing deck height by 20 cm did not alter behavioural outcomes in weaner pigs during either 8- or 23-h journeys

(Kobek-Kjeldager et al., 2025). Together, these findings indicate that increasing vertical space does not necessarily result in greater behavioural use of that space or improved welfare during transport.

A similar distinction between available space and its actual behavioural use was observed for stocking density. As expected, unused floor space increased as stocking density decreased. However, rabbits at LOW frequently remained grouped while part of the crate remained unoccupied, particularly during transit. Thus, reducing stocking density did not necessarily result in greater spatial dispersion within the crate. Similar observations have been reported in pigs, where increasing space allowance did not necessarily increase lying behaviour and, under some conditions, greater freedom of movement was associated with disturbance and difficulties maintaining balance during vehicle motion (Rioja-Lang et al., 2019). Importantly, this does not imply that higher stocking densities are preferable, but rather that the relationship between stocking density and welfare is not necessarily linear and depends on animal and transport conditions.

These findings highlight that the evaluation of space requirements during transport should consider not only the theoretical area required to accommodate particular postures and how animals actually use the available space under commercial conditions, but also whether providing additional space results in measurable welfare benefits, taking into account factors such as environmental conditions and transport duration.

Thermophysiological response

Following the 6-h fasting period on farm, rabbits had a mean body weight of 2.44 ± 0.11 kg, falling within the commercial slaughter weight range in Spain (2.0 -2.5 kg). Before crating, 95.8% of rabbits were normothermic and 4.2% were classified as hyperthermic, while no cases of hypothermia were recorded. The presence of hyperthermia at this stage warrants consideration, as these cases may reflect individual variation in RT or transient physiological responses to pre-transport handling (weighing and RT measurement). Infrared thermography may offer a less invasive alternative, as lacrimal temperature has shown a strong correlation with RT in rabbits Contreras-Jodar et al. (2025b).

After 5 h 20 to 6 h of transport duration, rabbits had lost an average of 75 g, equivalent to 3.1% of their initial body weight. Individual BW loss ranged from 0 to 160 g, indicating high inter-individual variability in BW loss. BW loss during transport is likely to reflect several processes, including losses through urine and faeces, water loss, and mobilization of body reserves (Jolley, 1990). Mean BW loss was higher than the 60.7 g recorded under the summer transport conditions evaluated by Contreras-Jodar et al. (2026). Nevertheless, neither stocking density nor crate height affected significantly BW loss in either study. In the summer study, the fixed effects explained only a small proportion of the variance in BW loss ($R^2_m = 0.029$), increasing to $R^2_c = 0.172$ when the randoms effects were considered (subzone nested within the batch). A similar pattern was observed in the present study ($R^2_m = 0.048$; $R^2_c = 0.243$), suggesting that differences in stocking density and crate height within the ranges evaluated were not major determinants of BW loss and that other sources of variability contributed more strongly to the observed inter-individual differences. Crate location within the truck was subsequently explored as a potential source of this

variability and the model including crate location through fixed effects also explained very little variance ($R^2_m = 0.098$; $R^2_c = 0.311$). Thus, the observed variability in BW loss appeared to be driven primarily by batch-to-batch differences rather than by crate treatments or spatial location within the vehicle.

A similar lack of effect of the crate treatments was observed for RT. Mean RT decreased by 0.6 °C after transport, although individual changes ranges from -2.5 to 0.6 °C, indicating high inter-individual variability in the thermal response. This decrease was accompanied by a shift towards hypothermia. Normothermia remained the predominant thermal state across all crate treatments (79.2–93.8%), but hypothermia was detected in every crate treatment, ranging from 6.2 to 20.8%. Neither stocking density nor crate height affected the change in RT or the probability of hypothermia. Crate treatments explained only a small proportion of the variability in the change in RT ($R^2_m = 0.062$), with the explained variance increased to $R^2_c = 0.127$ after accounting for the random effects. A similarly low contribution was observed under summer conditions ($R^2_m = 0.039$; $R^2_c = 0.052$; Contreras-Jodar et al., 2026), indicating that stocking density and crate height explained little variation in RT response under either seasonal scenario. However, the direction of thermal response differed between seasons: in summer, both hypothermia (7.3%) and hyperthermia (5.9%) were recorded after transport, whereas in autumn the thermal response shifted towards hypothermia in 12.8% of rabbits.

Unlike BW loss, however, the variation in RT change showed a clear spatial pattern within the truck. When crate location was considered, the fixed effects explained 35.1% of the variation ($R^2_m = 0.351$; $R^2_c = 0.443$), with longitudinal position having a significant effect. RT decreased progressively more

from the rear (-0.3 °C) to the middle (-0.6 °C) and front (-0.9 °C) of the vehicle, whereas vertical and side position had no significant effects. Thus, under the conditions of the present study, longitudinal location within the truck appeared to be a considerably more important determinant of the thermal response than stocking density or crate height.

This shift towards lower RT in the present study occurred despite in-crate temperatures remaining above external ambient temperature throughout most of the transport process. Temperatures inside the crates were within the range of approximately 12–25 °C during catching and loading and decreased once the vehicle was in motion to 10–22 °C, while differences among truck subzones persisted during transit. The increase in air movement after departure may have contributed to convective heat loss, while the spatial variability in the in-crate microclimate indicates that rabbits were not exposed to identical thermal conditions throughout the vehicle. Nevertheless, environmental measurements alone do not fully explain the thermal response at the individual level, as heat exchange may also be influenced by physical contact with conspecifics, posture and local airflow.

Wetness was assessed because urine soiling may have thermoregulatory consequences in rabbits during transport. Intentional urination onto conspecifics during transport has been previously described by Finzi (1991) who also reported RT values approximately 1 °C lower in wet rabbits. Similarly, Contreras-Jodar et al. (2025b) observed a high prevalence of hypothermia among urine-soiled rabbits kept at approximately 21 ± 2 °C and $68.6 \pm 11.0\%$ RH, particularly at the lowest stocking density evaluated (~ 55 kg/m²), comparable to the LOW treatment of the present study, whereas this response was less pronounced at higher

stocking densities ($\sim 70\text{--}83\text{ kg/m}^2$) comparable to the present MED and HIGH treatments. Together, these observations suggest that urine-soiled fur may increase heat loss under certain microclimatic conditions.

The relationship between stocking density and wetness itself, however, was also not consistent across seasonal studies. Under summer conditions, wetness was most prevalent at LOW, whereas in the present autumn study the highest prevalence occurred at MED. Crate height did not affect wetness in either study. The lack of consistent stocking density-related pattern suggests that urine soiling is unlikely to be determined by available space alone.

Despite the occurrence of hypothermia after transport and lairage, no lethargic rabbits were observed in any of the crate treatments. This contrasts with observations under controlled climatic conditions where sustained thermal exposure for 8 h was accompanied by lethargic responses in some rabbits showing both hypo- and hyperthermia (Contreras-Jodar et al., 2025b). In the present study, therefore, the decrease in RT observed in some rabbits was not accompanied by overt signs of severe thermal compromise. Mortality was also absent among the subset of rabbits individually assessed in both batches, while DOA at truck level was 0.04% (one rabbit per truck). Slaughterhouse records from Spain, France and Italy similarly indicate DOA rates ranging from 0.0 to 0.2% (Contreras-Jodar et al., unpublished data). Petracci et al. (2010) reported an overall DOA rate of 0.09% in commercially transported rabbits in Italy and found increased mortality at external temperatures below 7.3%, with mortality also increasing when journey duration exceeded 205 min, whereas Voslášřová et al. (2018) similarly reported greater mortality with increasing transport distance.

Overall, the autumn transport conditions resulted in a shift towards heat loss in some rabbits, but without evidence of progression towards more severe welfare consequences. Together with our previous findings under summer conditions (Contreras-Jodar et al., 2026), these results indicate that stocking density and crate height within the ranges evaluated explain little of the variability in the thermophysiological response of rabbits contrasting seasonal transport conditions.

Metabolic response

The metabolic indicators assessed (haematocrit, glucose, LDH, CK, NEFAs and CORTI) were selected to evaluate hydration status, energy mobilisation, physical exertion, and activation of the stress response during transport. Collectively, these metabolic indicators provide complementary information on the physiological cost associated with the transport process (Knowles and Warris, 2000), including in rabbits (De la Fuente et al., 2004; Verga et al., 2007; Trocino et al., 2018).

Consistent with the thermophysiological results, stocking density, crate height and their interaction explained very little of the total variability ($R^2_m = 0.011$ – 0.066). In contrast, including truck subzone nested within the batch as a random effect substantially increased the explained variance for glucose ($R^2_c = 0.453$), NEFAs ($R^2_c = 0.236$) and CORTI ($R^2_c = 0.438$). Therefore, the potential contribution of truck position to the variability observed in these responses was further explored.

Glucose concentrations were higher in rabbits transported at the front of the truck (176 mg/dL) than in those located in the middle (155 mg/dL) or rear (150

mg/dL), whereas no differences were detected according to vertical or side position. Interestingly, this pattern paralleled that observed for RT, as rabbits at the front also showed the greatest decrease in RT. CORTI showed a similar longitudinal tendency, although the effect did not reach statistical significance, with higher concentrations at the front than at the rear (23.2 vs. 19.9 ng/mL). These concordant longitudinal patterns coincide with marked spatial differences in the transport environment. During transit, the front of the truck was generally exposed to greater air speed, with peaks above 4 m/s in both batches, while air speed was lower in the middle and negligible in the rear. The highest vibration exposure was also consistently recorded in the upper-front subzone, and noise levels were highest towards the front of the vehicle.

Greater air speed at the front may have enhanced convective heat loss and contributed to the greater decrease in RT observed in this area, whereas the combined exposure to airflow, vibration and noise may have contributed to the spatial differences in metabolic and stress-related responses. However, rabbits at the front of the truck were among the first to be crated and loaded and the last to be unloaded and uncrated, resulting in a longer crating period and, at the time of sampling, a longer fasting duration and greater cumulative exposure to the overall transport process. This may be particularly relevant for glucose, as plasma glucose responses in transported rabbits have previously been reported to depend on prior feed availability and time in transit (Jolley, 1990). Therefore, the relative contributions of microclimatic conditions, mechanical exposure and duration of confinement cannot be determined from the present data.

The relevance of transport conditions beyond stocking density is also supported by previous studies in rabbits. De la Fuente et al. (2004) found no

effect of stocking density on glucose, cortisol, CK or LDH, whereas several of these variables differed between summer and winter transport conditions. Similarly, Liste et al. (2008) reported spatial differences in glucose, corticosterone and CK according to position within the truck, although the pattern differed from that observed here. Together with the present results, these findings indicate that environmental and operational conditions experienced during commercial rabbit transport, including spatial heterogeneity within the vehicle, can be important determinants of physiological responses and may, under some conditions, outweigh the effects of stocking density.

In contrast to glucose and CORTI, NEFAs showed a spatial pattern according to vertical rather than longitudinal position, with higher concentrations in rabbits transported in the lower than in the upper part of the truck (0.597 vs. 0.541 mmol/L). As NEFAs reflect the mobilisation of adipose reserves, this difference may indicate some spatial variation in energy mobilisation within the vehicle. However, no clear vertical pattern was identified in the environmental conditions recorded during transport, and the relatively small difference observed warrants caution in attributing this response to a specific mechanism.

Haematocrit was unaffected by stocking density or crate height, providing no indications of differences in hydration status among crate treatments. The absence of crate treatment effects on LDH and CK is consistent with the behavioural observations, as rabbits remained predominantly immobile during transit irrespective of the space available. Thus, there was little behavioural evidence that differences in stocking density or crate height resulted in sufficiently different levels of muscular activity to produce detectable changes in these indicators. The considerable individual variability observed, particularly for CK

and LDH, further suggests that factors unrelated to the experimental crate treatments contributed substantially to these responses.

The absence of metabolic effects of stocking density and crate height is also consistent with our previous findings under both controlled climatic conditions and commercial summer transport (Contreras-Jodar et al., 2025b, 2026). Thus, across markedly different environmental conditions, these factors have consistently explained little of the metabolic variability within the ranges evaluated.

Overall, the metabolic responses provide no evidence that reducing stocking density or increasing crate height resulted in measurable physiological benefits under the conditions evaluated. Instead, the spatial patterns observed for glucose and RT, together with the longitudinal tendency observed for CORTI and the vertical effect on NEFAs, suggest that spatial heterogeneity in environmental, mechanical and operational conditions within the vehicle may have been more relevant to some physiological responses than the crate treatments themselves.

Limitations

Several limitations should be considered when interpreting the present findings. First, the study was conducted under commercial conditions and involved a limited number of transport batches. Consequently, batch- and journey-specific factors, including journey duration, loading and unloading sequence, and external weather conditions among others, could not be experimentally controlled or separated from each other. This is particularly relevant to the spatial differences observed within the truck, as longitudinal

position was simultaneously associated with differences in confinement duration and exposure to environmental and mechanical conditions. Therefore, although consistent spatial patterns were identified for some physiological responses, the relative contributions of individual factors such as airflow, vibration, noise or duration of confinement cannot be established.

Second, the results obtained regarding stocking density and crate height are restricted to the ranges and transport conditions evaluated. The absence of measurable benefits from reducing stocking density or increasing crate height under the present conditions does not imply that these factors would be irrelevant under more challenging environmental conditions, different journey durations, animal weights or vehicle configurations. Their effects may interact with environmental and operational conditions particularly under thermal extremes.

Third, metabolic indicators were assessed only after transport, preventing within-individual changes from baseline to be quantified. Although paired pre-transport measurements would have strengthened the interpretation of these responses, blood collection itself requires handling and venipuncture and could have elicited an additional stress response before transport, potentially affecting subsequent measurements.

Conclusions

Under the Mediterranean autumn transport conditions evaluated, reducing stocking density from 87–89 kg/m² to 50–51 kg/m² and increasing crate height from 20 to 40 cm did not result in measurable welfare indicators assessed. Rabbits made limited use of the additional space provided: greater crate height

998 did not promote upright postures, while additional floor space did not necessarily
999 result in greater spatial dispersion within the crate.

1000 The study also revealed substantial spatial and temporal heterogeneity in
1001 the conditions experienced within the truck, including temperature, RH, air speed,
1002 vibration, and noise exposure. In-crate conditions differed dramatically from
1003 external ambient conditions, and truck subzone was associated with some
1004 physiological responses, particularly changes in RT and glucose concentrations.
1005 This highlights the importance of considering the microclimatic and operational
1006 conditions experienced by rabbits within the truck, rather than relying solely on
1007 external environmental conditions or theoretical space requirements.

1008 Overall, the present findings provide empirical evidence that, within the
1009 ranges, transport durations and environmental conditions evaluated, reducing
1010 stocking density or increasing crate height according to current recommendations
1011 did not provide detectable welfare benefits. These findings indicate that the
1012 assessment and monitoring of rabbit welfare during transport should not rely
1013 solely on compliance with theoretical planimetric requirements or the provision of
1014 sufficient space to accommodate specific postures, but should be supported by
1015 outcomes from animal-based welfare indicators. In addition, the environmental
1016 conditions experienced by rabbits during transport should be considered as
1017 relevant risk factors for welfare.

1018 1019 **Data and model availability statement**

1020 None of the data were deposited in an official repository. The data that support
1021 the findings of this study are available from the authors upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to correct the English language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of interest

None.

Acknowledgements

The authors are grateful to the rabbit integrator company involved in the project, as well as to their staff, for their collaboration and support during on-farm sampling and data collection at the slaughterhouse. The authors would also like to thank Berta Baulida, Mireia Campoy and Marilys Rendón from IRTA for their valuable technical work during the study.

1047

1048 **CRedit authorship contribution statement**

1049 A. Contreras-Jodar: Conceptualisation, Methodology, Writing – original draft,
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1053 Supervision. A. Velarde. Funding acquisition, Writing – review & editing,
1054 Supervision.

1055

1056 **Financial support statement**

1057 This work was supported by a grant from the Spanish Ministry of Agriculture,
1058 Fisheries and Food (Ministerio de Agricultura, Pesca y Alimentación) through the
1059 Innovative Project “WELFARECUN – New fattening, transport and slaughter
1060 processes in rabbit production to improve animal welfare”, funded under the
1061 European Innovation Partnership for Agricultural Productivity and Sustainability
1062 (EIP-AGRI), within the framework of the Spanish CAP Strategic Plan (PEPAC)
1063 for 2023.

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 1200

1201 **Table 1**

1202 Ethogram of the posture and behaviour of rabbits in the crate.

Posture/Behaviour	Definition
Upright standing	Rabbit bearing weight on hind legs, body elevated, either supported on another rabbit or not.
Stacking	Rabbit with the whole body on top of other rabbits (100% overlap).
Immobile	Rabbit does not move its body or head, groom, explore, interact socially, or attempt to escape.
Movement	Rabbit performing locomotor activity, including displacements or hops within the crate.
Extended ears	Head or ears in contact with the crate ceiling in 20-cm-high crates, or ears extending more than 20 cm above the floor in 40-cm-high crates.
Others	Other behaviours not included in the ethogram.

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Table 2

Proportion of behaviours expressed by rabbits across transport phases, stocking densities and crate heights during commercial transport. Behaviours were recorded by scan sampling every 5 min during three phases: 1h pre-transit (loading), 1h transit (vehicle motion) and 1 h post-transit (unloading and lairage).

Crate treatments			Posture/Behaviour, %					
Transport phase	Stocking density	Crate height	IMB	STK	ER	MOV	SU	OTH
Pre-transit	LOW	H20	77.8	0.0	3.2	0.0	19.0	77.8
		H40	68.3	1.9	3.7	1.7	24.4	68.3
	MED	H20	77.1	0.0	1.0	0.0	21.9	77.1
		H40	74.0	0.4	4.1	2.8	18.7	74.0
	HIGH	H20	75.2	0.0	1.2	0.0	23.6	75.2
		H40	73.8	8.2	5.0	4.6	8.4	73.8
Transit	LOW	H20	99.5	0.0	0.5	0.0	0.0	99.5
		H40	98.2	0.8	0.0	0.0	0.0	98.2
	MED	H20	99.4	0.0	0.6	0.0	0.0	99.4
		H40	99.0	0.0	1.0	0.0	0.0	99.0
	HIGH	H20	89.8	0.0	0.0	0.0	10.2	89.8
		H40	93.5	4.3	2.0	0.2	0.0	93.5
Post-transit	LOW	H20	90.0	1.2	5.0	0.0	3.8	90.0
		H40	89.8	0.0	0.0	0.0	10.2	89.8
	MED	H20	85.5	0.0	0.0	0.0	14.5	85.5
		H40	92.3	0.0	0.0	0.0	7.7	92.3

HIGH	H20	95.0	0.0	0.0	0.0	5.0	95.0
	H40	83.8	4.0	0.5	0.0	12.1	83.8

Abbreviations: LOW = 50–51 kg/m²; MED = 72–73 kg/m²; HIGH = 87–89 kg/m²; H20 = 20 cm; H40 = 40 cm; IMB = immobile; STK = stacking; ER = extended ears; MOV = movement; SU = standing up; OTH = other behaviours.

Table 3

Body weight, rectal temperature, thermal state and lethargic state of fattening rabbits after 6 h of fasting and immediately before being randomly allocated to the different crate treatments.

Stocking	Crate	N	BW, g	RT, °C	Thermal state			
					Hypothermia	Normothermia	Hyperthermia	Lethargy
LOW	H20	48	2.45	39.6	0 (0.0%)	46 (95.8%)	2 (4.2%)	0 (0.0%)
	H40	48	2.46	39.7	0 (0.0%)	46 (95.8%)	2 (4.2%)	0 (0.0%)
MED	H20	48	2.34	39.5	0 (0.0%)	44 (91.7%)	4 (8.3%)	0 (0.0%)
	H40	48	2.32	39.6	0 (0.0%)	47 (97.9%)	1 (2.1%)	0 (0.0%)
HIGH	H20	48	2.44	39.6	0 (0.0%)	44 (91.7%)	0 (0.0%)	0 (0.0%)
	H40	48	2.44	39.7	0 (0.0%)	47 (97.9%)	3 (6.2%)	0 (0.0%)

Abbreviations: LOW = 50–51 kg/m²; MED = 72–73 kg/m²; HIGH = 87–89 kg/m²; H20 = 20 cm; H40 = 40 cm; N = number of rabbits assessed; RT = rectal temperature.

Hypothermia was defined as rectal temperature <38.6 °C, normothermia as 38.6–40.1 °C, and hyperthermia as >40.1 °C, in accordance with Kahn and Line (2010).

Table 4

Prevalence of wet rabbits, lethargy, and thermal states (hypothermia, normothermia, hyperthermia) after transit and lairage at the slaughterhouse under different stocking densities and crate heights.

Stocking density	Crate height	N	Prevalence, %				
			Wet	Lethargy	Hypothermia	Normothermia	Hyperthermia
LOW	H20	48	25.0 _{ab}	0.0	4 (8.3%)	44 (91.7%)	0 (0.0%)
LOW	H40	48	18.8 _{ab}	0.0	7 (14.6%)	40 (83.3%)	1 (2.1%)
MED	H20	48	31.2 _{ab}	0.0	10 (20.8%)	38 (79.2%)	0 (0.0%)
MED	H40	48	35.4 _b	0.0	8 (16.7%)	40 (83.3%)	0 (0.0%)
HIGH	H20	48	12.8 _{ab}	0.0	5 (10.6%)	42 (89.4%)	0 (0.0%)
HIGH	H40	48	8.3 _a	0.0	3 (6.2%)	45 (93.8%)	0 (0.0%)

Abbreviations: LOW = 50–51 kg/m²; MED = 72–73 kg/m²; HIGH = 87–89 kg/m²; H20 = 20 cm; H40 = 40 cm.

Hypothermia was defined as rectal temperature <38.6 °C, normothermia as 38.6–40.1 °C, and hyperthermia as >40.1 °C, in accordance with Kahn and Line (2010).

Different subscripts in the same column indicate statistically significant differences (P<0.05).

Table 5

Metabolic and stress markers in fattening rabbits after transport and lairage at the slaughterhouse under different stocking densities and crate heights.

Item	N	LOW		MED		HIGH		SE	p-value				R ² m	R ² c
		H20	H40	H20	H40	H20	H40		SD	CH	SD×CH			
Haematocrit, %	24	38.3	38.5	37.5	37.8	38.1	38.2	0.3	0.075	0.402	0.956	0.040	0.069	
Glucose, mg/dL	24	160	162	160	162	164	155	4	0.823	0.548	0.089	0.023	0.453	
LDH, U/L	24	332	314	280	280	242	212	32	0.010	0.527	0.893	0.066	0.066	
CK, U/L	24	1509	1543	1702	1774	1463	1681	170	0.417	0.432	0.847	0.019	0.023	
NEFAs, mmol/L	24	0.569	0.593	0.573	0.599	0.534	0.550	0.029	0.176	0.316	0.984	0.026	0.236	
CORTI, ng/mL	24	20.5	22.1	22.1	21.9	20.6	21.7	1.3	0.670	0.344	0.637	0.011	0.438	

Abbreviations: LOW = 50–51 kg/m²; MED = 72–73 kg/m²; HIGH = 87–89 kg/m²; H20 = 20 cm; H40 = 40 cm; LDH = lactate dehydrogenase; CK = creatine kinase; NEFAs = non-esterified fatty acids; CORTI = corticosterone.

The mixed model included the stocking density (SD), the crate height (CH) and the interaction between SD and CH (SD×CH). Subzone within the truck, nested within batch was included as a random effect to account for spatial variability.



Figure 1. Transport vehicle and crate arrangement used in the two experimental batches. The vehicle was loaded with plastic transport crates arranged in 16 trolleys, with 8 or 10 crates stacked at different vertical levels.

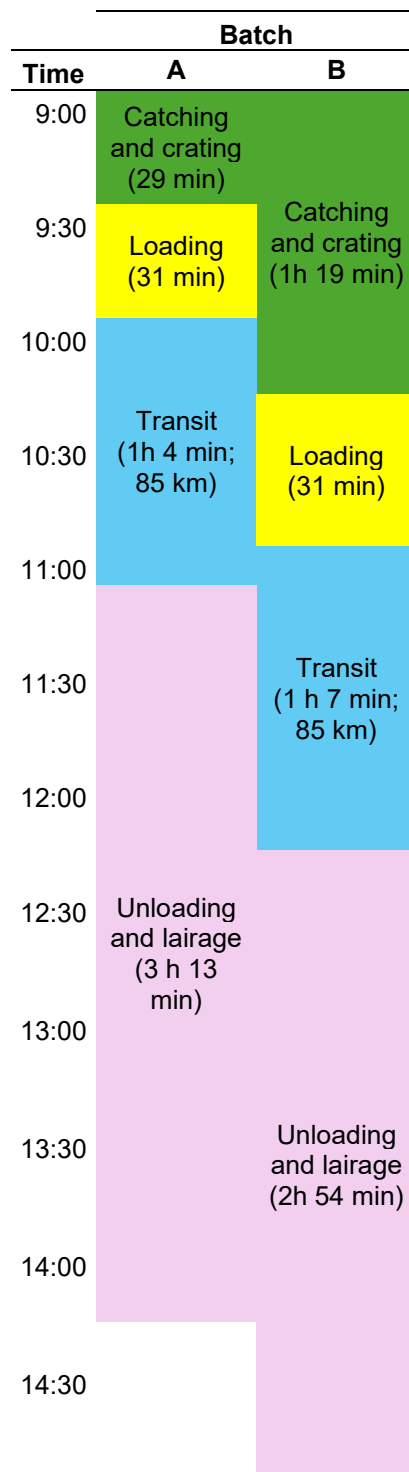


Figure 2. Schedule, duration and distance of the two commercial rabbit transports (A – B) to the slaughterhouse.

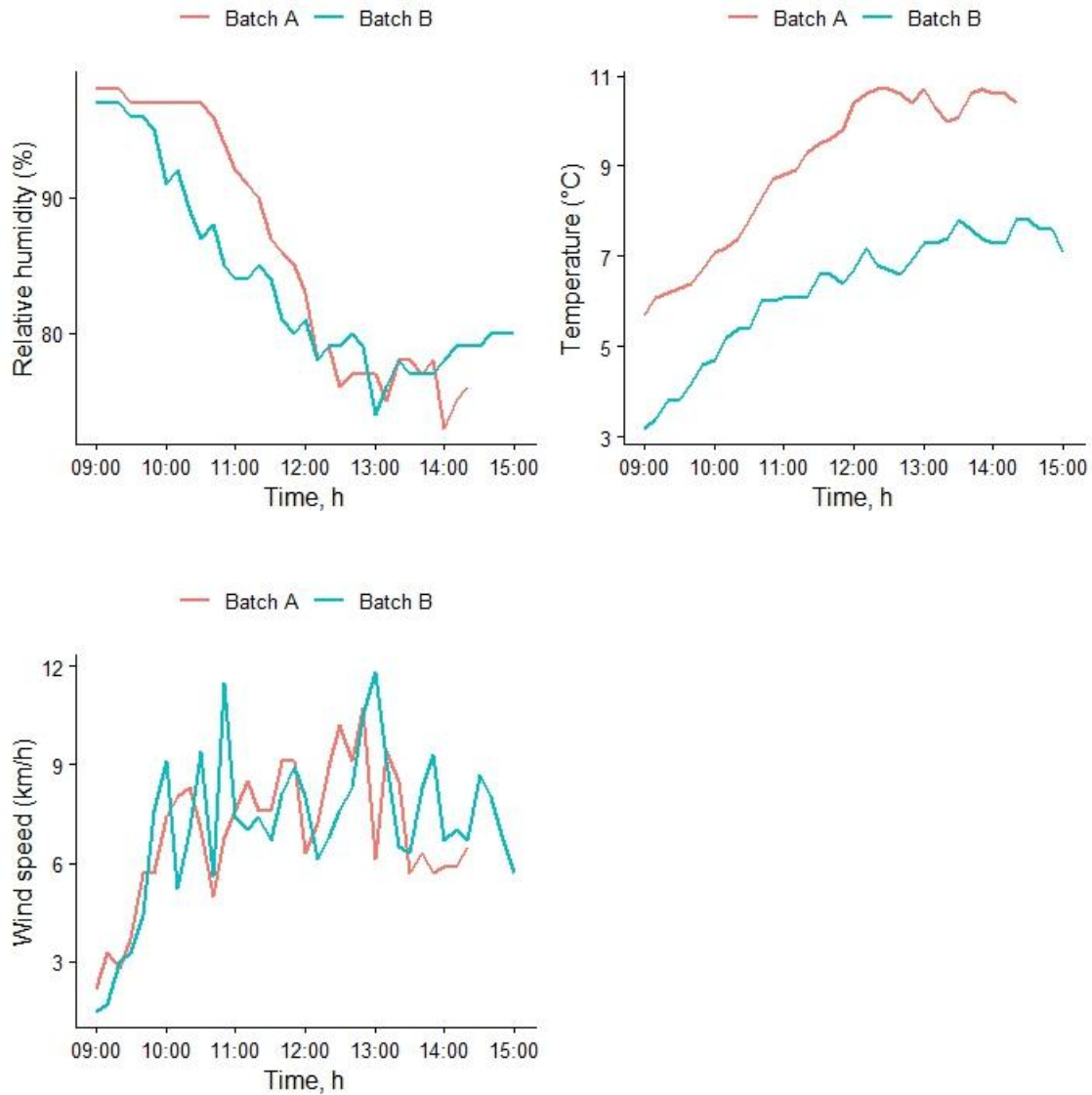
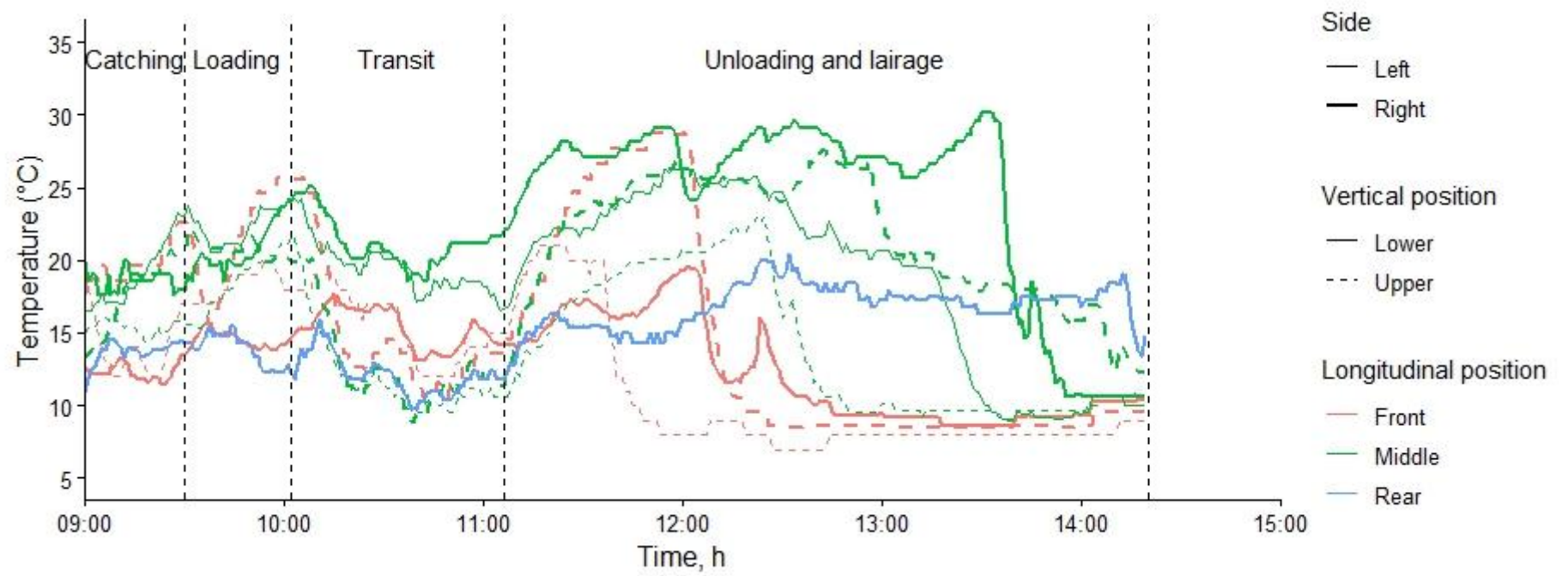
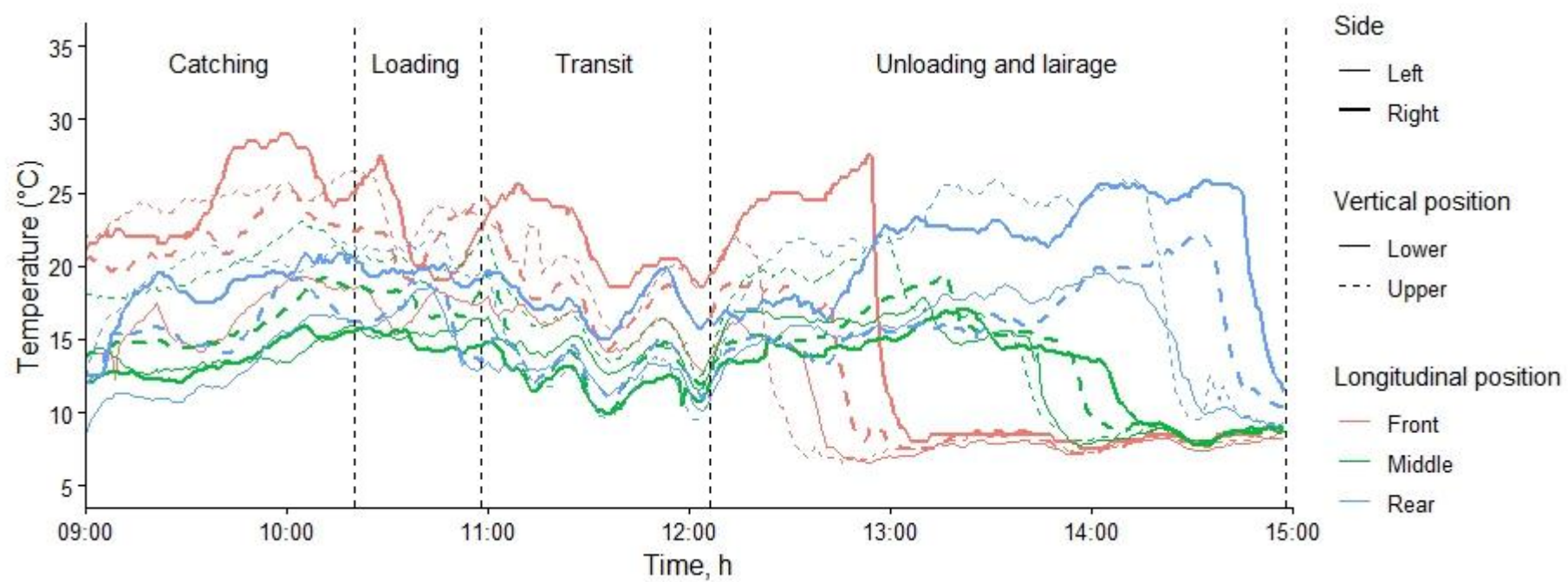
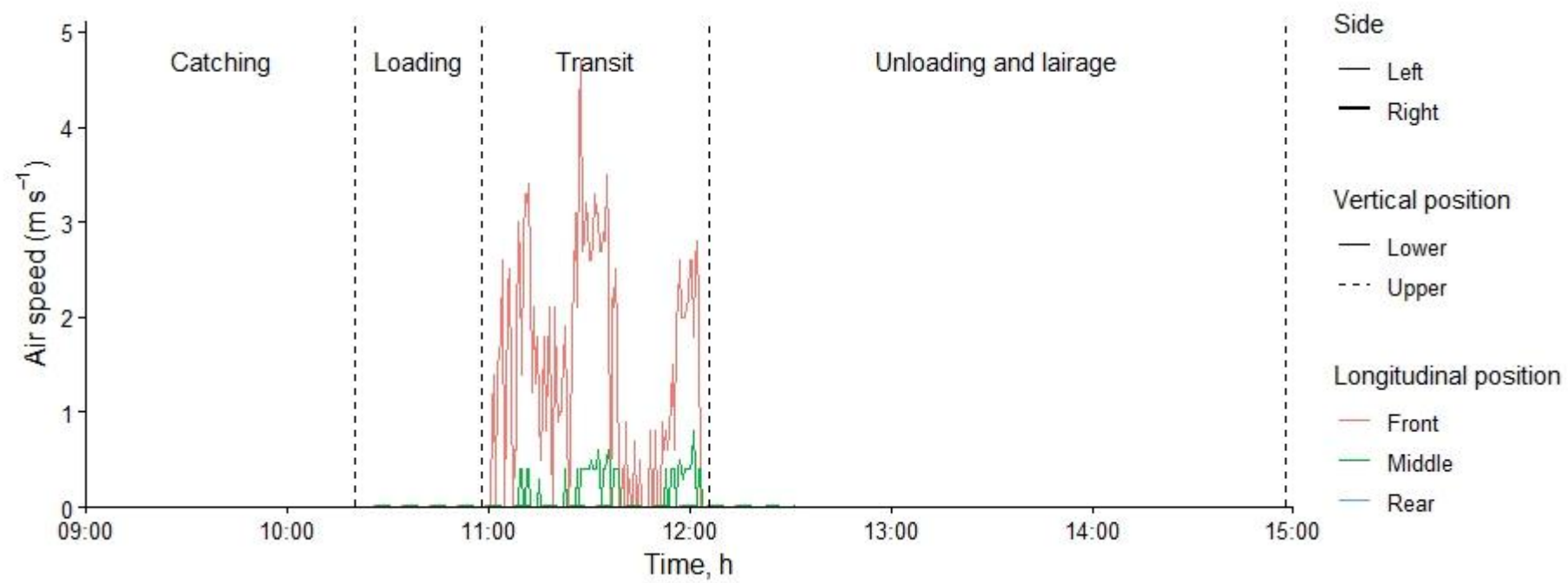
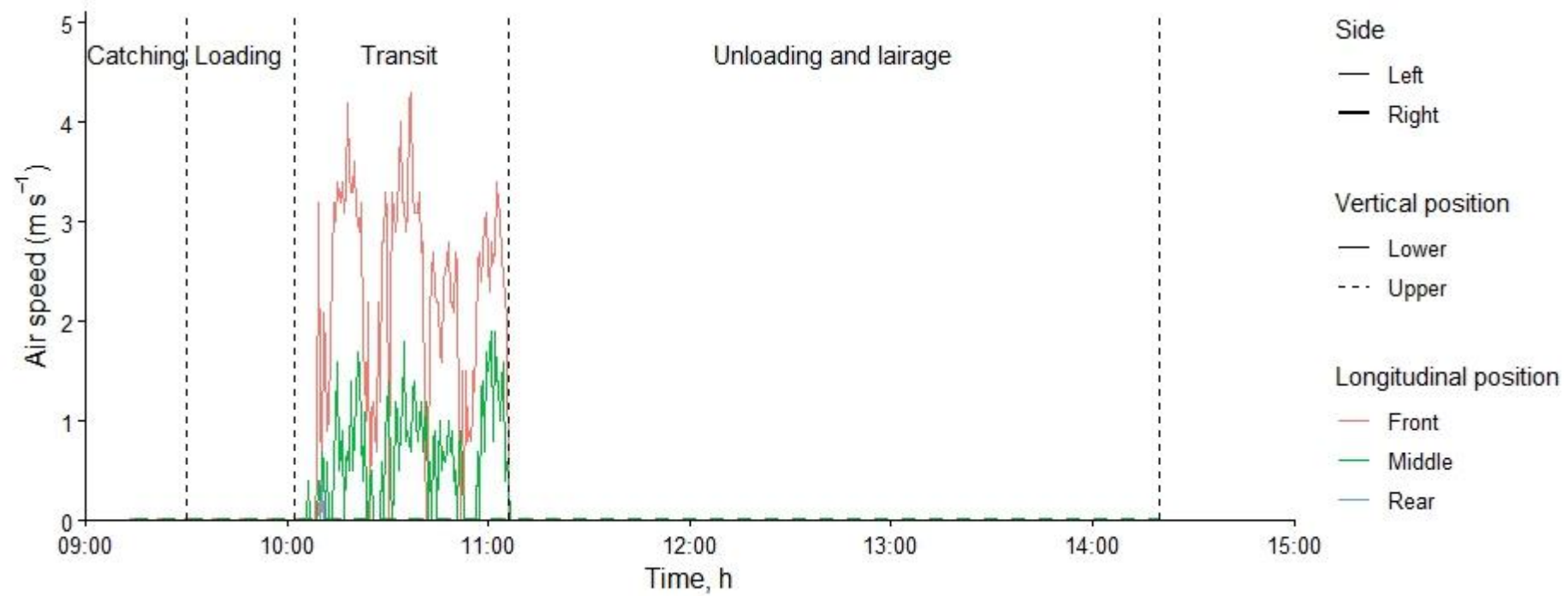


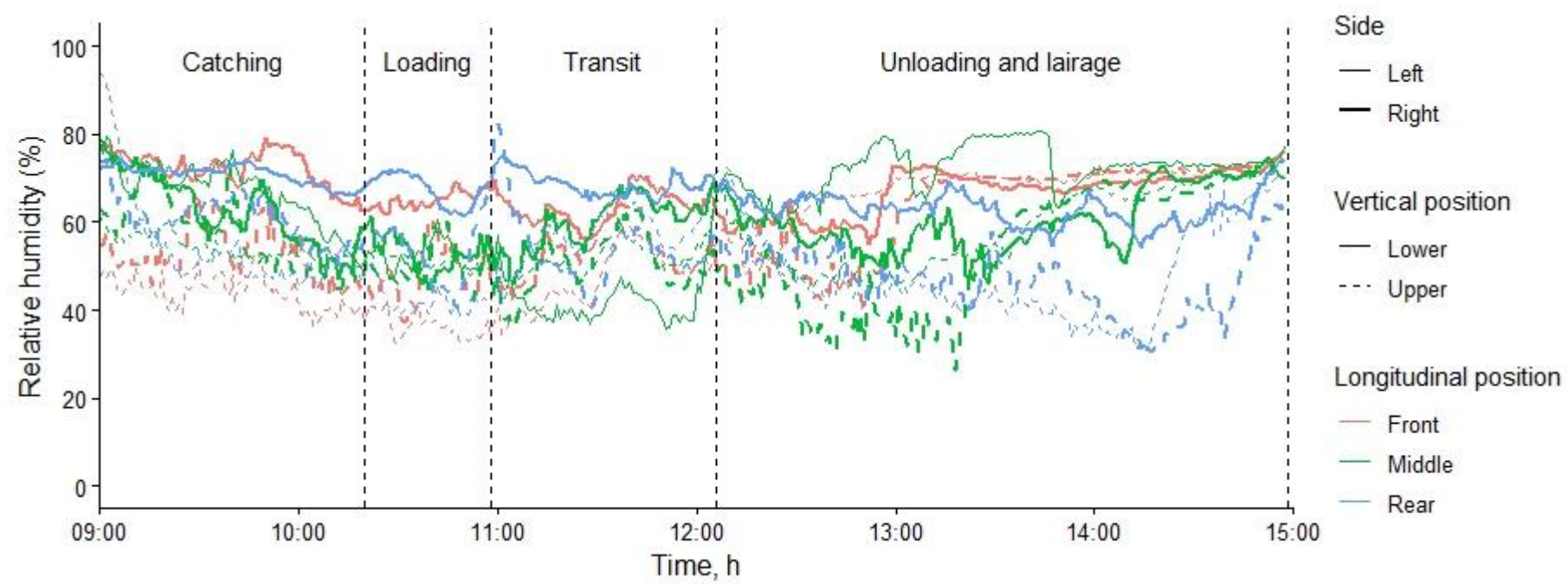
Figure 3. Evolution of ambient environmental conditions during the two commercial rabbit transports (batch A and B) obtained from the nearest meteorological station. A) temperature, B) relative humidity, and C) wind speed. No rainfall was recorded during either batch.











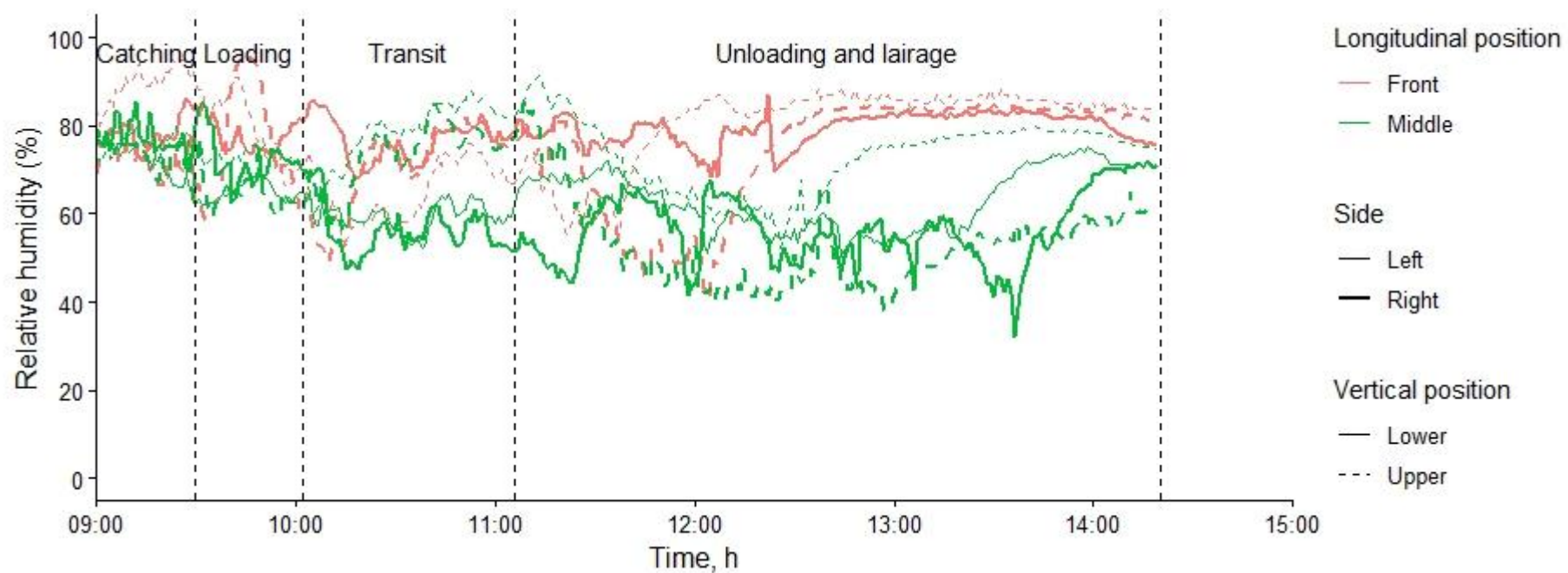


Figure 4. Temporal changes within the crate in A) temperature, B) relative humidity, C) air velocity, and D) vibration magnitude across the different truck subzones during two commercial rabbit transports to the slaughterhouse (batches A–B).

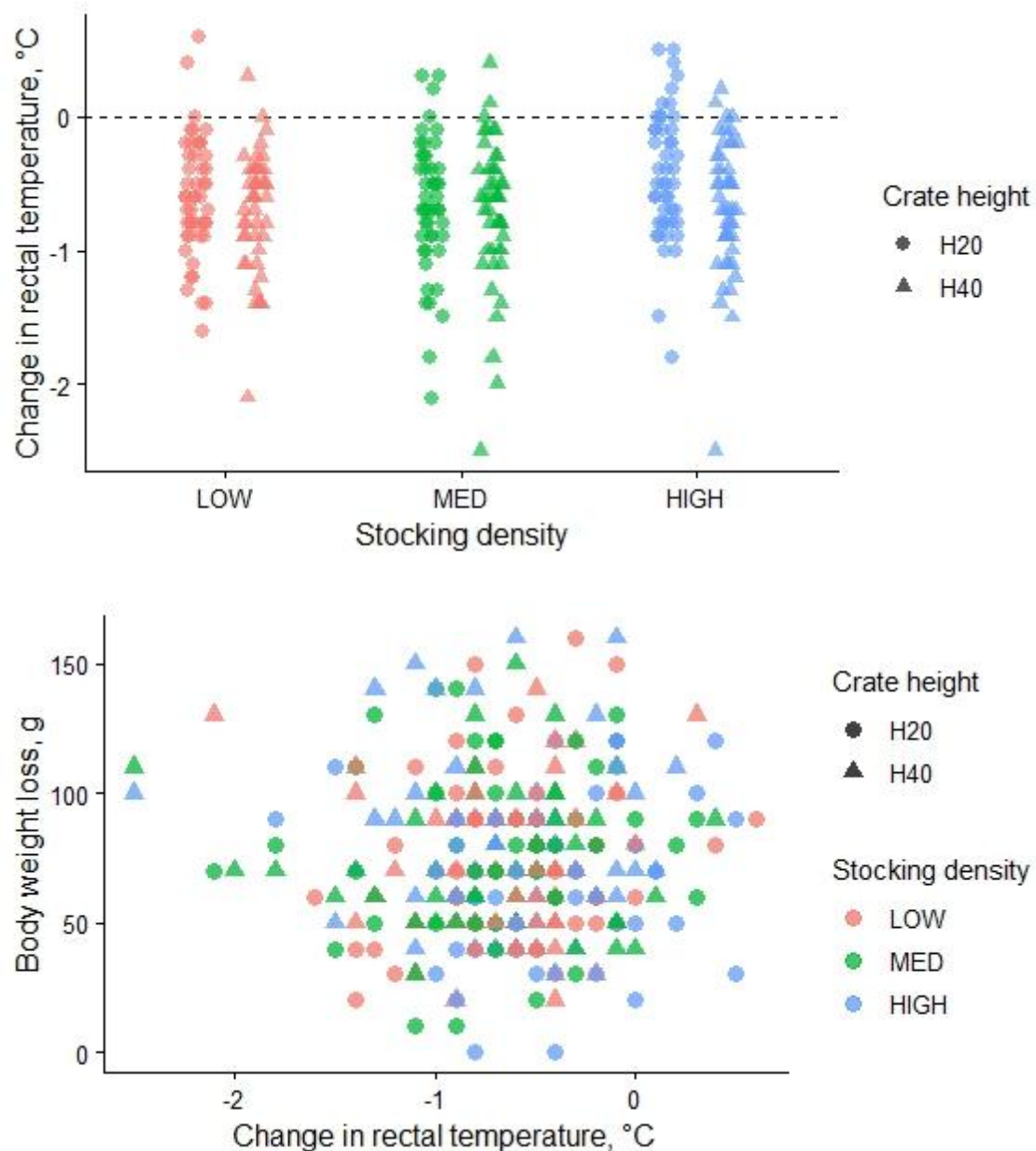


Figure 5. Thermophysiological response of rabbits following commercial transport according to stocking density and crate height. A) Change in rectal temperature. The dashed line indicates no change in rectal temperature. B) Relationship between body weight loss and change in rectal temperature. Each point represents an individual rabbit.

Supplementary material

