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Impact of pre-slaughter transport conditions on stress response, carcass traits, and meat quality in growing rabbits

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ABSTRACT

In growing rabbits (n = 320; 84 d of age), an increase in the journey duration to the slaughterhouse from 1 h to 3 h under mild climatic conditions (10–13 °C; 75–90% relative humidity) affected several stress indicators in the plasma and muscle collected at slaughter (cortisol; corticosterone; lactate dehydrogenase, LDH; lactate; heat shock protein 70 kDa, HSP70; osmolality, and Na) ($0.001 < P < .05$), reduced muscle L*, b* ($P < .01$), and meat shear force ($P < .05$), and increased the dressing out percentage and muscle pH ($P < .01$). An increase in the lairage duration before slaughtering from 30 min to 3 h affected muscle cortisol and plasma creatinine kinase, LDH, lactate, and HSP70 ($0.001 < P < .05$), increased dressing out percentage and muscle pH ($P < .05$), but decreased meat shear force ($P < .001$). The interaction between journey and lairage duration was significant for several stress indicators and meat quality.

1. Introduction

The transportation process, which includes catching, loading, travelling, unloading, and lairage, is a stressful experience that affects animal welfare (Broom, 2005). Animals respond to the stressors faced during transport by making behavioral, immune, hematological, and metabolic changes (Broom & Fraser, 2015; Warris, 2010), which may impair slaughter performance, and carcass and meat quality (Schwartzkopf-Genswein et al., 2012).

Scientific information about the relationships between transport conditions, animal stress, and product quality is relatively limited for rabbits compared to other species. Rabbits reared for meat production are usually only transported at the end of the production cycle when they are moved from the farm to the slaughterhouse. They are only commercially produced in a few countries within Europe (mainly Italy, Spain, and France) and slaughterhouses are usually located in high animal density areas. Many authors have previously investigated the effects of different factors, such as journey and lairage duration (Dal Bosco, Castellini, & Bernardini, 1997; Lambertini, Vignola, Badiani, Zaghini, & Formigoni, 2006; Liste et al., 2009), season (De la Fuente, Salazar, Ibáñez, & González De Chavarri, 2004; Liste et al., 2008; María et al., 2008), environmental conditions (De la Fuente, Díaz, Ibáñez, & González De Chavarri, 2007), crate distribution in the truck (Liste et al., 2008, 2009), and space availability (Accorsi et al., 2017) on rabbit stress and/or product quality. However, most of these studies usually tested one transport factor and a small number of indicators and they were not run under commercial conditions.

In Italy, where there is a large, commercially integrated chain, Petracci, Bianchi, and Cavani (2008) found that pre-slaughter management of rabbits was adequate and the integrated system had few weaknesses. However, in Spain, a survey about rabbit transport to slaughterhouses identified several critical points. These were the waiting time at the farm before loading, the loading process, ventilation and temperature during transport, unloading, lairage duration, and associated environmental conditions (Buil, María, Villarroel, Liste, & López, 2004).

This study investigated how crate distribution within the truck and journey and lairage duration influenced animal stress by measuring primary and secondary stress biomarkers. The study also investigated how these factors affected slaughter results, and carcass and meat quality in a controlled rabbit transport system from a commercial farm to the slaughterhouse.

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2. Materials and methods

All procedures and animal care were in compliance with Council Regulation (EC) n. 1/2005 on the protection of animals during transport, and Council Regulation (EC) n. 1099/2009 on the protection of animals at the time of killing.

2.1. Animals and experimental arrangement

In November 2015, a total of 2690 crossbred rabbits (84 d old; 3.0 kg BW) from a commercial farm were loaded into standard plastic crates (100 cm length × 50 cm width × 30 cm height). There were 12 animals per crate (space allowance: 139 cm^2/kg). One single truck authorized for animal transport, equipped with a rigid roof and open-side walls, transported the rabbits to a commercial slaughterhouse. During transport, the ambient temperature ranged from 10.1 °C to 13.4 °C and the humidity from 74.7% to 89.7% (Environmental Protection Agency of Veneto Region, Italy, meteo-station database).

At loading, 32 crates were assigned to 16 experimental groups, with two crates and 24 animals per group, in a four-factor experimental arrangement. These factors were crate distribution inside the truck (top vs. bottom; external vs. internal), journey duration (short vs. long), and lairage time (short vs. long). The longer journey and lairage times in this study were based on current slaughterhouse practices and corresponded to normally accepted durations. The top crates were the highest in the crate pile within the truck and the bottom crates were the lowest ones. The external crates were those exposed to air flow during the journey; and the internal crates were those located in the middle of the truck and were not exposed to air.

At the slaughterhouse, the short-journey crates were unloaded after a 1-h journey (48-km distance). However, the long-journey crates remained on the truck during the stop for unloading (30 min), and then were transported for a further 1 h and 30 min to be unloaded after a 3-h journey (120-km distance). Thus, the long journey tested in the present study consisted of two trips with one stop between them. This is a common situation in the commercial practice when one single truck loads rabbits from different farms. The short and long-journey group crates were also subjected to a short lairage (30 min) or a long lairage (3 h) time, respectively, before slaughtering in the lairage area next to the slaughter conveyor. Then, rabbits (320 rabbits; 10 rabbits × 32 crates) were individually taken out of crates, stunned, and hung on the slaughter conveyor according to standard procedures. Before stunning, rabbits were weighed in a quick and safe manner to avoid any stress or difference with commercial conditions. Stunned animals were individually identified by hanging a mark on the conveyor hooks.

2.2. Commercial slaughter and carcass and meat quality recordings

The animals were stunned by electro-anesthesia and killed by throat slitting, according to current slaughterhouse practices. After a 3-h cooling period at 3–4 °C, all the carcasses were weighed to calculate individual dressing out percentages.

A subset of 160 carcasses (5 rabbits × 32 crates), which were representative in terms of average weight and variability, was stored at 4 °C for further analysis. After 24 h, the pH of the *longissimus lumborum* (LL) and *biceps femoris* (BF) muscles was recorded in duplicate with a pH meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat.5232; Crison Instruments Sa); along with the L*a*b* color indexes (Commission International de l'Eclairage, 1976), which were measured in duplicate using a Minolta CM-508C spectrophotometer (Minolta Corp., Ramsey, NJ, USA).

The head, liver, thoracic organs, and kidneys were removed from the chilled carcasses to obtain the reference carcass (Blasco & Ouhayoun, 1996). Then, the reference carcass was dissected and the dissectible fat (scapular, inguinal, and perirenal) was removed. The hind legs and LL muscles were stored under vacuum in plastic bags at −20 °C. Later, the thawing and cooking losses for both cuts were measured. After thawing, the samples were put in plastic bags and cooked in a water bath until they reached an internal temperature of 80 °C, i.e., 1 h for LL and 2.5 h for the hind leg. After they had been left to cool for 1 h, a 7-cm section was cut from the intermediate part of LL and a TA.HDI dynamometer (Stabel Micro System Ltd., Godalming, UK) with an Allo-Kramer (10 blades) probe (load cell: 100 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 500 mm/min) was used to measure the maximum shear force (N) (Bianchi, Petracci, Pascual, & Cavani, 2007). The hind legs were cooled for 1.5 h and then the femur was removed and the portion corresponding to the BF muscle, which also included other muscle bundles, was weighed and its shear force was measured. All carcass and meat quality analyses were performed by blinded observers.

2.3. Stress biomarker analyses

Immediately after throat slitting, blood samples were taken from 160 rabbits (5 rabbits × 32 crates) and placed into lithium-heparin tubes. The plasma was directly obtained by centrifugation at 4033 rpm for 10 min (4 °C), and then divided into two fractions. One fraction was immediately stored at −20 °C and used to measure heat shock protein 70 kDa (HSP70) expression. The other fraction was stored at 4 °C and used to measure the other stress indicators. Two muscle samples were taken from the LL cranial part of the same 160 animals after the carcass had been refrigerated for 3 h. These samples were then stored at −20 °C until they were needed for the glucocorticoid analyses and lipid peroxidation measurements.

Haematocrit levels (%) were measured immediately after sampling by putting blood into capillary tubes and centrifuging them at 12500 rpm for 5 min (MicroCL 17, Thermo Scientific, Schwerte, Germany). Plasma glucose (mmol/l), urea (mmol/l), total proteins (g/l), sodium (Na) (mmol/l), and creatinine kinase (CK) and lactate dehydrogenase (LDH) activities (mmol/l) were determined in the plasma by a COBAS C501 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and commercially available kits (Roche Diagnostics GmbH). Lactate (mmol/l) was measured by a colorimetric enzymatic assay kit (Randox Laboratories Ltd., Ardmore, UK) and COBAS C501 analyzer. Finally, osmolality was calculated as follows:

$$\text{osmolality (mOSM/kg)} = 2 \times [\text{Na}] + [\text{urea}] + [\text{glucose}]$$

Plasma expressions of HSP70 (kpixels) were measured by western blot followed by a densitometry analysis (Negrato et al., 2013) after the protein concentration had been evaluated by a BCA protein assay kit (Thermo Pierce, IL, USA).

Cortisol and corticosterone levels (ng/ml) in the plasma and muscle were extracted according to Bertotto et al. (2010) and Trocino et al. (2014), and measured by microtiter radioimmunoassay (RIA) using species-specific antibodies (Biogenesis, Poole, UK) according to Simontacchi, Marinelli, Gabai, Bono, and Angeletti (1999). Parallelism, recovery, and intra- and inter-assay precision tests were performed to validate the RIA cortisol and corticosterone measurements in all the matrices. Optimal parallelism between the standard and diluted extract curves and reproducibility (CV < 0.10) data were obtained. The results showed that the extraction efficiency was > 70%.

Muscle lipid peroxidation was assayed by measuring the thiobarbituric acid-reactive substances (TBARS) according to Yoshida et al. (2005). The TBARS formation was expressed as the number of micromoles of malondialdehyde equivalents formed per ml of muscle, which was measured using a spectrophotometer at 535 nm (V-630, Jasco Europe S.R.L., Cremella, Italy). All stress biomarkers analyses were performed by blinded observers.

2.4. Statistical analysis

The number of animals per experimental group was defined