



Effects of stunning with different carbon dioxide concentrations and exposure times on suckling lamb meat quality

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ABSTRACT

Forty-nine Manchega breed male suckling lambs were used to determine the effect of different stunning methods (using two different CO₂ concentrations and exposure times) on lamb meat quality. The lambs were allocated to five stunning treatments including four CO₂ treatments [80% CO₂ for 90 s (G1); 90% CO₂ for 90 s (G2); 90% CO₂ for 60 s (G3); 80% CO₂ for 60 s (G4)] and an electrically stunned control group (G5). The gas-stunning treatments did not cause neither haematomas nor blood splash in the carcasses. Meat quality was evaluated by testing pH, colour (L^* , a^* , b^* , chroma, hue values), water holding capacity (WHC), cooking loss (CL), shear force (SF), drip loss (DL) and total aerobic bacteria. Statistical differences in pH at 24 h post-mortem, colour, WHC and CL were not found among groups. After 7 days post-mortem, there were statistical differences among groups in pH (highest in G4 and G5) and in DL (highest in G1). There were differences in SF due to stunning method evident after 72 h and 7 days ageing. The statistical differences ($P < 0.01$) among groups on total aerobic bacteria at 24 h (lower and higher values in G2 and G5, respectively) disappeared at 7 days post-mortem. As G2 as G3, could be recommended to stunning suckling lambs since a highest stability with ageing time on meat quality was found using 90% CO₂.

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1. Introduction

Many advantages have been attributed to carbon dioxide (CO₂) stunning method because it require less handling of the animals, restraint during stunning is not necessary, and more than one animal can be stunned simultaneously (EFSA, 2004). Moreover, CO₂ stunning leads to improvements in worker safety since there is less kicking during exsanguination and shackling (Larsen, 1982). On the other hand slaughter men are more at risk with the electrical stunning due to the high voltages and the animal convulsions (EFSA, 2004).

In pigs, stunning with CO₂ is used due to its positive effects on meat quality as compared to electrical stunning (Velarde, Gispert, Faucitano, Manteca, & Diestre, 2000). Channon, Payne, and Warner (2000) indicated a lower incidence of ecchymosis and bone fractures, along with an improvement in both meat quality and worker safety with gas-stunning in pigs compared with electrical stunning. In sheep, the most widely used system is electrical stunning. However, according to Vergara and Gallego (2000), this conven-

tional method causes the meat to age more quickly. Vergara, Linares, Berruga, and Gallego (2005) indicated greater tenderness, lower drip losses and no blood splash in gas-stunned lambs. Later, Linares, Bórnez, and Vergara (2008) concluded that stunning with CO₂ gas could be a valid alternative to prevent the negative effects of electrical stunning systems on meat quality characteristics. Although the EU Council Directive 93/119/CE (1993) stated that the concentration of CO₂ for stunning pigs must be at least 70% by volume, this directive does not stipulate the gas concentration/exposure time in other species such as sheep. It is therefore necessary to determine the most suitable combination (CO₂ concentration and exposure time) to reduce stress and suffering in animals and optimise meat quality in sheep.

The aim of the present study was to evaluate the influence of different stunning procedures (using two different CO₂ concentrations and exposure times) on the meat quality of suckling lambs.

2. Materials and methods

The experimental protocol was approved by the University of Castilla-La Mancha Animal Ethics Committee, according to the Executive Committee Directive 86/609/CEE of 2 November 1986 regarding the protection of animals used in research and for scientific purposes.

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2.1. Animals and experimental design

Forty-nine Spanish Manchega male suckling lambs were used in this trial. All animals were raised exclusively on milk and slaughtered at 12.80 ± 0.20 kg live weight (30 days old).

Lambs were distributed into five groups according to the type of stunning:

- Four groups were stunned with gas using different CO₂ concentrations and exposure times [G1: 80% CO₂ for 90 s ($n = 9$); G2: 90% CO₂ for 90 s ($n = 10$); G3: 90% CO₂ for 60 s ($n = 10$); G4: 80% CO₂ for 60 s ($n = 10$)] using a gondola dip-lift system (G van Wijnsberghe & Co n.v. Veurne, Belgium) normally used for stunning pigs (3 m long \times 1.5 m wide \times 1 m high). The concentration of CO₂ in the pit was tested by authorized personnel. Animals were placed in a gondola in groups of 3 or 4. The motor on the gondola transporter was set to reach the bottom of the pit in 10 s and to the ejection level 16 s after the conclusion of the exposure period. The lambs were bled by cutting the blood vessels of the neck between 25 and 35 s after removal from the gondola.
- A control group [G5 ($n = 10$)] was electrically stunned at 110 V, 50 Hz for 5 s (plate electrodes applied on both sides of the head, behind the ears; Electronarcosis Panel, MAC-01, Bernard, S.L.).

After stunning, lambs were slaughtered and dressed using standard commercial procedures. Then, carcasses were subjected to visual examination in order to determine the incidence of both external bruises (haematomas) and blood splash (ecchymosis). Finally, carcasses were chilled at 4 °C for 24 h in a conventional chiller.

2.2. Analysis of samples

Meat quality measurements were assessed on the *Longissimus dorsi*. First, pH was measured using Crison 507 (Crison Instruments S.A.) equipment with a penetrating electrode, at 0 and 45 min after the end of the dressing procedures (pH₀, pH₄₅). After 24 h post-mortem, the muscle was removed from the carcass and two pieces from the T7 to T11 in both sides of carcasses. One of them was used to evaluate the initial meat quality (at 24 h post-mortem). The other sample was packed in a clear tray (LINPAC, Plastic, West Yorkshire) with an oxygen permeability rate of $3.2 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$ at 1 atm and 23 °C, and covered by a film (having an oxygen permeability of $500 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$ at 1 atm and 25 °C). Then sample was analysed after 7 days of storage at 2 °C.

At 24 h and at 7 days the following measures were assessed:

Before to remove the muscle of the carcass pH was measured at 24 h post-mortem (pH₂₄) and after at 7 days (pH₇).

- *Water holding capacity* (WHC), as a percentage of free water (Grau & Hamm, 1953).
- *Colour coordinates* (L^* : lightness; a^* : redness; b^* : yellowness values), using a chromameter Minolta CR400 (Konica Minolta Sensing, Inc.) according to the method described by Vergara, Molina, and Gallego (1999). Also, chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue [$h^* = \arctan(b^*/a^*)$] values were calculated. These measurements were taken within ten minutes after having opened the package.
- *Microbiological analysis*: meat samples (approximately 5 g) were transferred to a sterile bag with 45 ml of tryptone phosphate water (buffered peptone water; Scharlau Chemie, Barcelona, Spain) and blended for 60 s in a Stomacher (Masticator, IUL Instruments, Barcelona, Spain). Duplicate 100 μ l inoculums of 10^{-1} decimal solution (primal solution) were spread on Petri dishes using a spiral system (Eddy-Jet, IUL Instruments, Barcelona), to determine total viable aerobic counts on plate count

agar (PCA, Scharlau Chemie S.L., Barcelona, Spain). Plates were incubated at 32 °C for 72 h. Microbial colonies from plates were counted using an automated colony counter (Counterstat Flash, IUL Instruments, Barcelona, Spain).

At 72 h and 7 days post-mortem the following parameters were assessed:

- *Cooking loss* (CL), expressed as the percentage of weight after cooking relative to the initial weight (just before the cooking). Meat samples (of approximately 40 g weight) were weighed and individually placed in polyethylene bags in a water bath at 70 °C for 15 min. After drying the cooked samples with filter paper they were weighed again.
- *Shear force* (SF), which was analysed using a TA.XT2 texture analyser equipped with a Warner–Bratzler device. For this analysis, the cooked meat samples, used for cooking loss measurements, were cut into three replicate pieces with a 1 cm² cross-section and 2–3 cm in length. SF was then recorded.
- *Drip losses* (DL) were analysed at 7 days post-mortem, expressed as a percentage of the initial portion weight (Vergara, Gallego, García, & Landete-Castillejos, 2003).

2.3. Statistical analysis

Data were analysed with the Statistical Package SPSS 14.0 (SPSS, Inc., Chicago, USA, 2005). The effect of different stunning procedures (G1–G5) on initial meat quality and after 7 days post-mortem was analysed using ANOVA. When the differences among groups were significant ($P < 0.05$), a Tukey's test at a significance level of $P < 0.05$ was carried out to test for differences between the treatment means. The differences in meat parameters due to time of storage (initial and after 7 days) were analysed using an ANOVA.

3. Results and discussion

The effects of the different gas-stunning procedures (different CO₂ concentrations, 80% or 90%, and exposure times, 60 or 90 s) on suckling lamb meat quality parameters are presented in Tables 1–4.

3.1. Incidence of carcass haematomas and ecchymosis

No gas-stunning procedure caused neither haematomas nor blood splash in the carcasses or meat. Gregory (2005) indicated that the gas stunning resulted in fewer blood spots in the meat and fewer haemorrhages on the surface of the carcass compared with electrical stunning. In our study, all carcasses from electrical stunning treatment showed both haematomas and petechial haemorrhages in agreement with Gilbert and Devine (1982).

3.2. pH

Stunning treatment did not affect pH at any time point (pH₀, pH₄₅ and pH₂₄) (Table 1). This is in agreement with other authors [Northcutt, Buhr, and Yung (1998), in turkeys; Vergara and Gallego (2000), in lambs; Önenç and Kaya (2004), in cattle]. However, differences in pH between the treatment groups were evident after 7 days storage ($P < 0.01$). The lowest pH value (5.65) was found in both G5 and G4 groups, while the highest pH was found in G2 (5.73). G1 and G3 groups had intermediate values. In contrast, Vergara and Gallego (2000) and Linares, Bórnez, and Vergara (2007) indicated a lower pH in electrically stunned lamb meat, in

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