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CO_2 stunning procedure on Manchego light lambs: Effect on meat quality

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ABSTRACT

This study examined the effect of different gas stunning methods (concentration of CO_2/time of exposure (G1: 80%90s; G2: 90%90s; G3: 90%60s; G4: 80%60s) on the initial meat quality of Manchego breed light lambs (25 kg live weight) and at 7 days post-mortem, assessed by pH, colour coordinates, water holding capacity (WHC), cooking loss (CL), drip loss (DL) shear force (SF) and lipid oxidation. An electrically stunned control group (G5) was used. Stunning method had a significant effect on pH values ($P < 0.001$) as well as on pH decline ($P < 0.01$). The lowest pH was found at 24 h post-slaughter in G1 and the highest one on G5. The greatest drop in pH (pH_0–pH_{24}) was found in G1 and G5 while the smallest in G3. In general values of colour coordinates, WHC and DL were similar in all groups. Stunning method affected CL ($P < 0.001$) at 7 days post-slaughter, with the lowest values being found in G1. Significant differences among groups were found ($P < 0.05$) in SF values at both post-mortem times, with less tender meat in groups stunned with 80% CO_2, especially in G1. A significant effect ($P < 0.001$) due to the type of stunning was found at 24 h on lipid oxidation, with the highest value in G5. After ageing this parameter was lowest ($P < 0.05$) in G1 and G4.

1. Introduction

EU consumers currently show a preference for foods produced in accordance with correct animal welfare standards, associating them with better quality and food safety (Kyprianou, 2007), and they also request transparency in pre-slaughter handling (Quintili & Grifoni, 2004). Stunning before slaughter is a legal requirement (EU Council Directive 93/119/EC) for attaining an adequate state of unconsciousness in animals previous to slaughter in order to avoid unnecessary suffering. A full report on aspects of the main stunning systems for the principal species of animals has been carried out by the EFSA, European Food Safety Authority (2004). For sheep, the most common method in Spain is electrical stunning. However, many disadvantages have been observed with the application of this method, e.g. animal welfare (Pearson, Kilgour, de Langen, & Payne, 1977) and carcass quality (Vergara, Linares, Berruga, & Gallego, 2005). According to Gregory (2005), the incidence of convulsions, fractures and muscle haemorrhages in carcasses is increased when using electrical procedures, often making stunning unadvisable. A previous study on lamb (Linares, Bórnez, & Vergara, 2008a) showed that CO_2 could be an alternative stunning method in order to prevent the negative effects of the electronarcosis system on ageing meat. Linares, Bórnez, and Vergara (2008b) concluded that further work is necessary to establish a correct interval for stunning and correct gas concentration in this type of lamb to obtain good meat quality. Moreover, the EU Council Directive 93/119/EC (1993) stated that the concentration of carbon dioxide for stunning pigs must be at least 70% by volume. However, there is no mention in this regulation for the gas concentration/time of exposure in other species such as sheep.

Although the effect of concentration/time exposure on suckling lambs (12–13 kg live weight) has been determined (Bórnez, Linares, & Vergara, 2009a), it has yet to be determined on the meat quality of light lambs, which is the type of meat preferred by habitual lamb consumers (Bernabéu & Tendero, 2005). This reason obliges us to determine the effect of CO_2 stunning procedure in light lamb based on meat quality.

Thus, the aims of the present study were (1) to evaluate the effect of different gas stunning methods on light lamb meat quality and (2) to determine the best combination of CO_2 concentration (80% and 90% volume in air) and exposure time (60 and 90 s) in order to obtain the best meat quality.

2. Materials and methods

The experimental protocol was approved by the University of Castilla-La Mancha Animal Ethics Committee, according to the

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Executive Committee Directive 86/609/CEE of 2 November 1986 regarding the protection of animals used in research and for scientific purposes.

2.1. Animals and experimental design

Fifty Spanish Manchega breed light male lambs from the flock at the Experimental Farm of Castilla-La Mancha University (Albacete, Spain) were used in this trial. Animals were slaughtered at 25 kg live weight (70 days old), having been fed with milk until weaning at 12 kg of weight (30 days old) and then with a commercial concentrate and cereal straw *ad libitum* until slaughter.

Lambs were transported from the farm to the slaughterhouse (20 km during approximately 30 min) in a vehicle adequately conditioned. Then, animals were placed in pens (3 m wide × 5 m long; 6–10 lambs per pen) and remained there during approximately 15 h, without access to food, although they received water *ad libitum*. After lairage, lambs were slaughtered and dressed using standard commercial procedures. Signs of unconsciousness were assessed from the moment of stunning to slaughter (Bórnez, Linares, & Vergara, 2009b), according to EFSA (2004).

Lambs were distributed into five groups ($n = 10$) according to the type of stunning:

- Four groups were stunned with gas using different CO_2 concentrations and exposure times, (G1: 80%90s; G2: 90%90s; G3: 90%60s; G4: 80%60s), with a gondola dip-lift system (G van Wijnsberghe and Co n.v. Veurne, Belgium) normally utilized for stunning pigs (3 m long × 1.5 m wide × 1 m high) that indicates the concentration of CO_2 in the pit, which was tested by the authorized personnel. Animals were placed in the box in groups of 3 or 4. The motor of the gondola transporter was set to reach the bottom of the pit in 10 s and to return to the ejection level 16 s after restarting. The lambs were stuck between 25 and 35 s after tipping the gondola, and were dressed 4 min later.
- A control group (G5) 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.) and immediately afterwards were stuck.

Immediately after stunning, lambs were slaughtered using standard commercial procedures. All carcasses were chilled at 4 °C for 24 h in a conventional chiller.

2.2. Sampling and analysis

The *Longissimus dorsi* muscle was used to measure meat quality. *pH* was assessed after dressing at 0 min (pH_0), 45 min (pH_{45}) and at 24 h post-mortem (pH_{24}) using a Crison 507 equipment with a penetrating electrode.

At 24 h post-mortem the *L. dorsi* muscle was removed from the carcass and divided into two pieces. One of them was used to evaluate the initial meat quality (at 24 h post-mortem) and the other was packed in a clear tray (LINPAC plastic) with a film (having an oxygen permeability of 500 cm^3 m^{-2} day^{-1} at 1 atm and 25 °C) and was then analysed after 7 days of storage (maximum time permitted for sale according to the requirement on its official label). Samples remained at 2 °C in a conventional chiller.

At 24 h post-mortem the following parameters were measured:

- Water holding capacity (WHC) as a percentage of free water (Grau & Hamm, 1953); *colour coordinates* (L^*, lightness, a^*, redness, b^*, yellowness values, chroma (C^*) and hue-angle (h^*) were calculated as $C^* = (a^* + b^*)^2)^{1/2}$ and $h^* = \tan^{-1}(b^*/a^*)$ using a chromameter Minolta CR400 according to the proposal by Vergara, Molina, and Gallego (1999).
- *Lipid oxidation*: TBARS content (as thiobarbituric acid reactive substances) was determined in duplicate from 5 g of *L. dorsi* muscle as described by Tarladgis, Pearson, and Dugan (1964). Absorbance at 532 nm was read with a Helios alfa spectrophotometer (THERMO, Electron Corporation, England). Results were expressed as mg MDA kg^{-1} of meat.

At 72 h post-mortem the following parameters were measured:

- *Cooking loss* (CL), expressed as the percentage of loss related to the initial weight (Vergara, Gallego, García, & Landete-Castillejos, 2003) followed by *shear force* (SF), which was analysed using a TA.XT2 texture analyser equipped with a Warner–Bratzler device. For this analysis, each meat sample was individually placed in a polyethylene bag in a water bath at 70 °C for 15 min. After drying the cooked samples with filter paper, they were cut into three replicates with a 1 cm^2 cross-section and 2–3 cm in length. SF was then assessed.

After 7 days post-mortem, all the above-mentioned parameters (pH, colour, WHC, CL, SF and lipid oxidation) were analysed again and also drip loss (DL), expressed as a percentage of the initial portion weight (Vergara et al., 2003).

2.3. Statistical analysis

The effect of different stunning treatments on initial meat quality and after 7 days post-mortem was analysed using an ANOVA procedure with the Statistical Package SPSS 14.0 statistical version (SPSS Inc., Chicago, USA, 2005). When the differences among groups were significant ($P < 0.05$), a Tukeýs test at a significance level of $P < 0.05$ was carried out to check the differences between pairs of groups. The differences in meat parameters due to time of storage (initial and after 7 days of storage) were analysed using an analysis of variance.

3. Results and discussion

3.1. pH values and pH decline

Significant differences ($P < 0.001$) among groups were found in pH_0, pH_{45} and pH_{24}, but disappeared at 7 days post-mortem (Table 1). A very low pH (5.36) was found in the G1 group (80% CO_2/90s) at 24 h post-slaughter, which could be due to a high glycolysis rate post-mortem. So, the plasma lactate level in G1 was higher than in the rest of the stunning groups, reaching values of 4.22 mmol/L (Bórnez et al., 2009b). This is in agreement with the results found by Nowak, Mueffling, and Hartung (2007) in pig subjected to the same type of stunning.

The higher the CO_2 concentration the shorter the induction to unconsciousness. 100% CO_2 induces unconsciousness rapidly; however, the induction to unconsciousness with this concentration is not instantaneous. On the other hand lower concentrations are far less effective and the insensibility is not instantaneous with the gas stunning method (Scientific Committee on Animal Health and Animal welfare, SCAHAW). This could cause some physiological responses in the animal causing variations in pH. So the lowest pH found in G1 group could be associated with the highest catecholamine values (Bórnez et al., 2009b).

On the other hand, it is assumed that the initial phase of the gas stunning causes aversion to CO_2 and many papers have studied this subject in different animal species such as turkeys (Raj, 1996); pigs (Raj, 1999; Raj & Gregory, 1995); poultry (Raj & Tserveni-Gousi, 2000 or in rats (Niel & Weary, 2007). However, this is not supported by other studies that concluded that higher CO_2 concentrations are more aversive than lower (Velarde et al., 2007).These