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Systems stunning with CO_2 gas on Manchego light lambs: Physiologic responses and stunning effectiveness

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ABSTRACT

Effect of four stunning treatments using different CO_2 concentrations and exposure times (G1: 80%90 s; G2: 90%90 s; G3: 90%60 s; G4: 80%60 s) on hormonal, haematological and biochemical parameters in Manchego breed light lambs and their stunning effectiveness (% animals correctly stunned) was studied. An electrically stunned control treatment (G5) was used. G1 showed the highest plasmatic hormonal, red cell distribution (RDW) and lactate levels. Haemoglobin, mean corpuscular volume (MCV), mean cell haemoglobin (HbCM), glucose, lactate deshydrogenase (LDH), sodium, potassium and creatine kinase (CK) were highest in G5. Stunning effectiveness was maximum (100%) in G3 and G5, only 50% in G1 and G2 and minimum (30%) in G4.

A discriminant analysis showed a function for discriminating between G5 and the gas stunned groups, and another one for discriminating between gas stunned groups. Only potassium and adrenaline variables marked the difference among groups.

1. Introduction

Stunning before slaughter is a legal requirement according to European law (EU Council Directive 93/119/EC, 1993) in order to ensure that animals do not suffer needlessly and are unconscious and insensible to slaughter procedure. The stunning method itself should be painless and as close as possible to instantaneous in its effect (Holst, 2001). Furthermore, it should provide duration of unconsciousness and insensibility, which ensures that death from subsequent slaughter, will intervene before recovery of sensibility (Cook, Devine, Travener, & Gilbert, 1992).

Electrical stunning is the most common method for sheep, involving transcranial application of an electric current of sufficient magnitude by using a pair of tongs (or electrodes) placed on both sides of the head, preferably on wet skin. To avoid painful electric shock due to wrong placement of the electrodes, sheep should be individually restrained manually in a trap or in a restrainer (EFSA (European Food Safety Authority), 2004). However electrical stunning can cause a cardiac dysfunction, circulatory arrest and increasing blood splash (Kirton, Frazerhurst, Woods, & Chrystall, 1981).

Carbon dioxide is commonly used to stun pig and poultry. Gas stunning is being promoted because (1) it results in fewer blood spots in the meat and fewer haemorrhages on the surface of the carcass (Gregory, 2005); (2) the time to return of consciousness can be controlled and thereby stun-stick interval can be controlled without compromising animal welfare (Holst, 2001); (3) requires less animal handling while allowing more animals to be stunned per hour than electrical stunning (Nowak, Mueffling, & Hartung, 2007) and also better safety for slaughter-men. In lamb meat, Linares, Berruga, Bórnez, and Vergara (2007) found a lower lipid oxidation with this method, and Vergara, Linares, Berruga, and Gallego (2005) found not blood spots in carcass, both studies compared with electrical system. Finally, Linares, Bórnez, and Vergara (2008a) concluded that CO_2 method could be considered as an alternative method to electrical stunning for lambs, but these same authors (Linares, Bórnez, & Vergara, 2008b) indicated that further work is necessary to establish the correct exposure time for stunning and concentration of this gas.

EU Council Directive 93/119/CE (1993) stated that the concentration of carbon dioxide for stunning pigs must be at least 70%, but it is well know that although 100% CO_2 induces unconsciousness rapidly, lower concentrations are far less effective and the insensibility is not instantaneous (Scientific Committee on Animal Health and Animal Welfare, SCAHAW). It could cause some physiologic responses in animal and an impact on meat quality. Moreover the normative no mention about the gas concentration/time of exposure in other species such as sheep, thus, the aims of the

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present study were (1) to evaluate the physiological responses (hormonal, haematological and biochemical parameters) on light lamb after gas stunning method, with different combination of CO_2 concentration (80% and 90% volume in air) and exposure time (60 and 90 s) and (2) to determine the effectiveness (% of lambs correctly stunned) of each stunning treatment.

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.

2.1. Animals and experimental design

One hundred-three Spanish Manchego breed light male lambs were used for this study. 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 distributed into five groups according to the type of stunning:

- Four groups were stunned with gas using different CO_2 concentrations and exposure times [G1: 80%90 s ($n = 20$); G2: 90%90 s ($n = 21$); G3: 90%60 s ($n = 22$); G4: 80%60 s ($n = 20$); volume in air]; in a gondola dip-lift system (G van Wijnsberghe & Co n.v. Veurne, Belgium) usually used for stunning pigs (3 m long × 1,5 m wide × 1 m high) that indicate the concentration of CO_2 in the pit, which was tested every experiment day 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 of the gondola, and were dressed 4 min later.
- A control group (G5; $n = 20$) 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.

Animals were slaughtered on ten days (replicate) and all factors such as transport and lairage conditions, and practices of people who handle lambs previous to stunning–slaughter were meticulously controlled to get identical conditions on experimental days.

2.2. Stunning effectiveness (S.E): percentage of animals correctly stunned

Signs of unconsciousness or death were controlled from the moment of stunning to slaughter in order to establish the percentage of animals that were correctly stunned according to EFSA (2004), so the lambs were classified into three groups: *dead* (absence of respiration, absence of pulse, absence of corneal and palpebral reflex and loss of hear function); *semiconscious* (only showed uncoordinated movements and they attempts to raise the head) and *correctly stunned* (animal is unable to respond to normal stimuli, including pain, but have breathing not-rhymic).

2.3. Blood sampling

Immediately after stunning, a blood sample from each animal was collected from the first sticking blood. Samples used for haematology determination were collected in 1 ml tubes containing EDTA (Ethylene Diaminotetraacetic Acid). Blood samples for hormonal and biochemical parameter concentration measurements were collected in 4 ml tubes without additives. Blood samples were maintained at 2 °C in a portable refrigerator until arrival at the laboratory for clinical analyses.

The following physiological parameters were analysed:

- **Haematological:** Haematites, Haemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Cell Haemoglobin (HbCM), Mean Cell Haemoglobin Concentration (CHbCM), Red Cell Distribution (RDW) and Leucocytes, were measured with an electronic haematological analyser (ABX Micros 60, Horiba ABX, France).
- **Hormonal:** Cortisol, Adrenaline and Noradrenalin. The determination of total Cortisol concentration was carried out through a competitive enzyme assay (EIA, RADIM, Pomezia, Italy). During the first incubation, the sample Cortisol competed with the Cortisol conjugated to horseradish peroxidase (HRPO) for the specific sites of the antiserum coated on the wells. Following the incubation, all unbound material was removed by aspiration and washing. The enzyme activity which was bound to the solid phase was inversely proportional to the Cortisol concentration in calibrators and samples, which was evidenced by incubating the wells with a Chromogen solution (Tetramethylbenzidine, TMB) in a substrate-buffer. Colorimetric reading was carried out using a spectrophotometer at 450 and 405 nm. The assay sensitivity was 5 ng/ml.

  The catecholamines were analysed using a competitive enzyme immunoassay kit [Cat Combi (Adrenaline/Noradrenalin) ELISA, EIA-4309, DRG Instruments GmbH, Germany]. The sensitivity in the analyses was 11 pg/ml for Adrenaline and 44 pg/ml for Noradrenalin.
- **Biochemical:** Glucose, Total Protein, Urea, Creatinine, Lactate Deshydrogenase (LDH), Creatine Kinase (CK) and Lactate, were measured utilizing a clinical system autoanalyzer (Synchron CX4 delta, Beckman Coulter, Inc.). A flame photometer (Corning model 435, Corning, England) was used to determine Sodium and Potassium plasma concentration.

2.4. Statistical analysis

Data were analysed with the Statistical Package SPSS 14.0 version (SPSS Inc., Chicago, USA, 2005). An ANOVA procedure was carried out to analyse the effect of the type of stunning on the levels of all blood parameters cited. When the differences among groups (G1 to G5) were significant ($P < 0.05$), a Tukey's test at a significance level of $P < 0.05$ was carried out to check the differences between pairs of groups.

A stepwise discriminant function analysis was carried out to select a linear combination of the independent variables that best allowed differentiating among the stunning methods. The analysis was defined by two canonical discriminant functions which were illustrated by means of a dispersion diagram.

3. Results

3.1. Haematological parameters

Table 1 shows the concentration of the plasmatic haematological parameters in the different stunning groups. There were found significant differences among groups in haemoglobin ($P < 0.05$), MCV ($P < 0.01$), HbCM and RDW (both $P < 0.001$) and leucocytes ($P < 0.01$) but not in the rest of haematological parameters.