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# Recovery of consciousness in hogs stunned with CO<sub>2</sub>: Physiological responses



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#### ABSTRACT

The objective of the present study was to determine the impact of recovering consciousness on physiological responses in hogs stunned with different concentrations of  $CO_2$ . A total of 1336 pigs were moved into a  $CO_2$  anaesthesia chamber for 90 s. The remaining pigs were assigned to 3 groups according to the  $CO_2$  concentration used for stunning: 85, 90 or 95%. Each group was then further divided into 2 sub-groups: those exsanguinated during the first 60 s after leaving the chamber without recovering consciousness (WRC); and those exsanguinated after more than 60 s that recovered consciousness (RC). The blood pH of the RC pigs decreased below 7.08, but their blood levels of  $Ca^{2+}$  (>1.59 mmol/L), glucose (>159.79 mg/dL), and lactate (>103.52 mg/dL) all increased when compared to reference values (RV) (P < 0.05). Therefore, a greater metabolic and energy imbalance occurs during exsanguination when pigs recover consciousness. In conclusion, exsanguination should be performed immediately upon the pigs leaving the  $CO_2$  chamber.

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#### 1. Introduction

Maintaining high standards of animal welfare during transportation (Becerril-Herrera, Mota-Rojas, et al., 2009; Mota-Rojas et al., 2014; Roldan-Santiago, Martínez-Rodríguez, et al., 2013) lairage, and slaughter requires appropriate equipment and supervision of employees (Roldan-Santiago, Mota-Rojas, et al., 2013; Yáñez-Pizaña et al., 2012). During slaughter, the animals should be unconscious in order to avoid inflicting undue pain and stress during the procedure; thus they should not be allowed to recover consciousness post-stunning (Grandin, 2003; Mota-Rojas et al., 2006, 2009). Changes in arterial and venous blood gas values have been described in swine during induction of CO<sub>2</sub> anaesthesia (Martoft et al., 2002), while Gregory, Raj, Audsley, and Daly (1990) affirm that carbon dioxide is unpleasant to breathe due to its acid

aroma, which can be uncomfortable at high concentrations and causes sensations of irritation and asphyxia, though this phenomenon only affects the animals in the early stages of anaesthesia. Most developed countries and many developing nations have laws that require stunning before slaughtering (FAO, 2001). Sacrifice without prior stunning is an extremely controversial practice from the standpoint of animal welfare (Grandin, 2010) because some animals killed without stunning take a long time to lose brain function and finally expire; a finding that has been shown experimentally using somatosensory-evoked responses in the brain, and on the basis of the delay before the animal collapses (Gregory, 2008). An additional implication is that the efficiency of exsanguination in animals slaughtered without stunning depends on the efficacious severing of the vagus nerves. Though subsequent bleeding efficiency and residual blood in the carcass have not been examined, it is well known that vagal severance affects blood pressure distribution and cardiac output responses during haemorrhaging. Welfare issues during slaughter without stunning include stress from the use of restraints, the pain caused by the cut itself, and the possibility

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that the animal may experience undue distress while bleeding out (Gregory, 2005). Stunning is used to produce insensibility by striking the animal or some other means (Becerril-Herrera et al., 2009; Mota-Rojas et al., 2010, chap. 16; Mota-Rojas, Hartung, Becerril-Herrera, & Bolaños-López, 2012, chap. 22). Stunning can be reversible or irreversible. In the first case, animals may recover consciousness before dying, such that the time between stunning and exsanguination is a determining factor for the efficiency of the stunning process. Irreversible stunning systems, in contrast, "stun" and kill the animal simultaneously. In this case, the objective of bleeding is to drain the blood from an already dead carcass, so the time factor is not critical from the standpoint of animal welfare (Quiroga & García, 1995; Velarde, Gisper, Faucitano, Manteca, & Diestre, 2000). Some researchers affirm that pigs should be exposed rapidly to 90% CO<sub>2</sub> (Becerril-Herrera, Alonso-Spilsbury, et al., 2009; Hartung, Nowak, Waldmann, & Ellerbrock, 2002), though it has been shown that exposure to CO<sub>2</sub> at 90% for 120 s is more effective than exposure for 90 s in abolishing corneal reflexes (Hartmann, Siegling-Vlitakis, Wolf, Rindermann, & Fries, 2009). The objective of the present study was to determine the behaviour of critical blood variables in pigs that recover consciousness after stunning with different concentrations of CO<sub>2</sub>.

#### 2. Methods

#### 2.1. Location

This study was carried out at a federal inspection plant in central

#### 2.2. Animals

A total of 1336 Mexican swine (between 105 and 110 kg of live weight) from a cross of Duroc sires with Yorkshire-Landrace sows were monitored. Animals fasted for 8 h prior to transport and were trucked to the abattoir with no access to food or water during an average travel time of 8 h. At the slaughterhouse, pigs were unloaded through a 0.95 m<sup>2</sup> (wide) by 10 m (long) fixed ramp with a 15° slope. The experiment was carried out in accordance with the guidelines for the ethical use of animals (Sherwin et al., 2003). All procedures related to the use and care of the animals strictly followed the Mexican regulation NOM-062-ZOO-1999. During loading and unloading at the farm and abattoir, respectively, the pigs were handled gently with no shouting. The pigs are moved using a type of plastic and wooden paddle, thus are directed toward different directions allowing or blocking access. No electric prods were used. Average ambient temperature and relative humidity upon arrival were 32–34 °C and 74%, respectively. During the 8-h rest period at the abattoir all pigs had access to water ad libitum, but not to food. They were housed with a space allowance of  $0.75 \text{ m}^2/100 \text{ kg during lairage}$ .

The pigs were classified into four groups: the first group rested in pens and were sampled 3 h before slaughtering (reference values, RV). For sampling of RV, a trained person restricted each pig by the snout with a rope and a second trained person obtained the blood sample by puncturing the jugular vein within 20 s using a syringe containing lithium heparin. The rest of the pigs were assigned to 3 groups according to the CO<sub>2</sub> concentration used for stunning: 85, 90 or 95%. Each group was then further divided into 2 sub-groups: 1) pigs stunned without recovering consciousness before bleeding (WRC); and animals that had recovered consciousness (RC). It is important to clarify that the researchers in this study did not manipulate in any way the time required for the pigs to recover consciousness; they simply followed the management routine at this abattoir. The velocity of the slaughtering line at the facility is very fast (280 pigs per hour) and the fact that some pigs become conscious post-stunning is a routine error that we took advantage of in order to study the phenomena explained above.

The pigs considered WRC were those that had a lapse between leaving the  $\rm CO_2$  chamber and exsanguination of less than 60 s. None of the hogs in this group recovered consciousness before bleeding. The other sub-group, classified as RC, included those animals that experienced a delay greater than 60 s between leaving the  $\rm CO_2$  chamber and bleeding, and that also presented re-establishment of respiration with positive palpebral and ocular reflexes before exsanguination (recovery of consciousness) (Table 1). The first breath was deemed to have occurred when thoracic movements were observed for the first time after stunning, followed by exhalation of air from the hog's muzzle. Also considered as an indicator of the absence of stunning was the emission of vocalizations upon leaving the  $\rm CO_2$  chamber.

#### 2.3. Assessment of the animals' blood parameters

Baseline blood samples were taken from the jugular vein using 0.25 mL heparinized syringes. The researcher who sampled the pigs had the skill required to collect the blood on the first attempt in less than 15 s. The blood samples taken post-stunning were collected during bleeding on an exsanguination table. Partial carbon dioxide [pCO<sub>2</sub> (mm Hg)] and oxygen [pO<sub>2</sub> (mm Hg)] pressure levels, as well as measurements of serum electrolytes [Na $^+$ , K $^+$  and Ca $^{2\,+}$  (mmol/L)], blood glucose (mg/dL), blood lactate (mg/dL), and haematocrit (%), were obtained using a blood gas and electrolyte analyser (GEM Premier, Instrumentation Laboratory Company, Lexington, USA, and Instrumentation Laboratory SpA Milano, Italy) (Becerril-Herrera et al., 2010).

#### 2.4. Stunning methods

The pigs were moved into a CO<sub>2</sub> stunner in groups of four. Handling was low-stress, as the animals were herded into the chamber by gentle slaps on their backs, with no shouting or blows, and without the use of electric prods or goads. The animals were stunned in a dip-lift CO<sub>2</sub> anaesthesia chamber set at atmospheres of 85, 90 and 95% CO<sub>2</sub> for 90 s. Four pigs were moved per gondola, and the depth of the exposure to gas was 3 m. This stunning facility consists of a runway equipped with a dip-lift unit. The runway from the lairage pens to the stunning units allows some movement but not enough to permit the animals to turn around. The floor of the cradle has holes to facilitate the distribution of the gas while the animals are at the end of the runway and in the cradle (Mota-Rojas, Becerril-Herrera, et al., 2012).

Immediately after stunning, the pigs were bled and the blood samples collected to assess the effect of stunning by analysing the acid-base balance and blood gases using the gas analysis equipment described above.

#### 2.5. Statistical analyses

Normality assays were performed (PROC UNIVARIATE/GLM SAS, 2007) for all the variables examined. In order to analyse the effect of stunning on the physiometabolic variables during recovery of consciousness, a general linear model ANOVA was applied (PROC GLM) (SAS Institute, Inc., 2004), which included the different concentrations of CO<sub>2</sub> employed to stun the hogs as the independent variables. The dependent variables were the levels of the partial carbon dioxide [pCO<sub>2</sub> (mm Hg)] and oxygen [pO<sub>2</sub> (mm Hg)] pressures, and measurements of serum electrolytes [Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> (mmol/L)], blood glucose (mg/dL), blood lactate (mg/dL), and haematocrit (%). The following equation was applied:

$$Yijk = \mu + \beta_1 X_1^{i} + \beta_2 X_2^{j} + \beta_3 X_3^{k} + \xi ijk$$

where: Yijk is the value of the dependent variables

 $\beta$  regression coefficient for factor 1  $\xi$ ijk Error.

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