International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 8 (3): 141-151, 2012
ISSN 1811-7775 / DOI: 10.3923/ijp.2012.141.151

Stunning Swine with CO_2 Gas: Controversies Related to Animal Welfare

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Abstract: The principal objective of this review is to characterize the physiological and biochemical events that occur in swine when stunned with carbon dioxide (CO_2) prior to slaughtering, as they relate to issues of animal welfare. Stress responses promote the maintenance of homeostasis and adaptation to the physiological and psychosocial challenges of a changing environment, a complex process that involves the coordinated activation of behavioural, autonomic and neuroendocrine reactions. When respired, CO_2 combines with water to form H_2CO_3 (carbonic acid), which generates elevated concentrations of H^+ ions that result in a state of acidosis at the cellular level. Today, questions have been raised concerning animal welfare, as some fear that inhaling high concentrations of CO_2 may cause distress in animals before they lapse into a state of unconsciousness. In particular, the bodily movements observed in the early phase of CO_2-induced anaesthesia in swine have led to concerns about stress. Pigs exposed to 80% CO_2 for 60 sec experience lactic academia and respiratory and metabolic acidosis. Lactate levels are an indicator of stress, while it has been shown that blood lactate concentrations associated with pre-slaughter stress factors (e.g., aggressive handling immediately prior to stunning) have detrimental effects on pork quality. From the perspective of animal welfare, 90% argon by volume, or the lowest possible CO_2 concentration necessary to stun swine, is recommended. Argon is suggested as a welfare-friendly alternative to carbon dioxide for stunning/killing pigs and poultry.

Key words: Animal welfare, carbon dioxide, stunning, pigs, stress

INTRODUCTION

Currently many researchers studying stress in animals from different approaches (Olmos-Hernandez *et al*., 2008; Vahdatpour *et al*., 2009; Ceylan *et al*., 2009; Dehghan *et al*., 2010; Yildirim and Yurekli, 2010; Hamidi *et al*., 2010; Mota-Rojas *et al*., 2011a; Martinez-Rodriguez *et al*., 2011a; Mota-Rojas *et al*., 2012a, b; Trujillo-Ortega *et al*., 2011). Maintaining high standards of animal welfare during transport and slaughter requires adequate equipment and strict supervision of employees (Becerril-Herrera *et al*., 2009a, b, 2010; Martinez-Rodriguez *et al*., 2011a, b; Mota-Rojas *et al*., 2011a, b, Mota-Rojas *et al*., 2012b; Roldan-Santiago *et al*., 2011). Also, animals should be unconscious at the time of slaughtering to avoid inflicting pain and stress during the procedure (Grandin, 2003; Mota-Rojas *et al*., 2010a, b; Orozco-Gregorio *et al*., 2010). Most developed countries and many developing ones, have laws that require stunning before sacrifice (FAO, 2001). According to Welfare Quality®, four principles must be respected to assure animal welfare: Food, comfort, health and the ability to express their natural behaviour. Today, initial efforts are underway to comply with the stipulations of this protocol (Mounier *et al*., 2010; Temple *et al*., 2011). Despite the current initiative to promote animal welfare, however, these four conditions are often still not met; due to inadequate knowledge, training and/or experience in animal handling (Gallo, 2008; Mota-Rojas *et al*., 2008; Mota-Rojas *et al*., 2011a, b;

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Becerril-Herrera *et al*., 2009b). In the case of swine, stress and inadequate animal welfare have been reported at different moments between farm and slaughterhouse; for example, during loading (Huertas-Canen, 2010; Grandin, 2010), transport (Mota-Rojas *et al*., 2006, 2009), unloading, herding, holding in pens (Mota-Rojas *et al*., 2009; Grandin, 2010; Smiecinska *et al*., 2011) and slaughtering (Becerril-Herrera *et al*., 2007, 2009a; Dalmau *et al*., 2010; Grandin, 2010; Mota-Rojas *et al*., 2010b).

The slaughtering of swine is carried out by slitting the arteries and veins of the brachiocephalic trunk to interrupt nutrient and oxygen supplies to the brain, thus causing death. Hence, any acceptable stunning system must first assure that the action does indeed render the animal unconscious quickly and without pain and second, that the unconscious state lasts until death (Quiroga and García, 1994; Mota-Rojas *et al*., 2008; Mota-Rojas *et al*., 2010a, b; Orozco-Gregorio *et al*., 2010). A stunning process produces insensibility by striking the animal or other means (Becerril-Herrera *et al*., 2009b). Despite the importance of this procedure, however, it seems that the information available on stunning prior to slaughter is still insufficient. Some authorities in different places insist that breathing blood into the upper respiratory tract and lungs causes suffering when slaughter occurs with no prior stunning (Gregory, 2008). Thus, it is extremely important that managers, veterinarians and scientists become more knowledgeable on how to assess and measure animal welfare at abattoirs (Grandin, 2010). To reduce pain and promote animal welfare at slaughter, a series of stunning methods have been designed and reported, including use of the captive bolt, electrical stunning (Grandin, 2010) and stunning with carbon dioxide (CO_2) (Hartung *et al*., 2002; Hartmann *et al*., 2009; Orozco-Gregorio *et al*., 2010). The latter method is now one of the most controversial and widely discussed.

In recent years, more and more scientific reports have emphasized that swine exposed to CO_2 during stunning experience aversion and stress. Many hog abattoirs that have adopted CO_2 stunning use a 90% concentration, while the minimum generally recommended concentration is 80%, a figure based on work originally conducted in Germany. More recent work, also from that country, indicates that the 90% concentration causes less physical stress than the lower amount, as it results in a less pronounced metabolic acidosis; however, 90% CO_2 can cause behavioural aversion (Nowak *et al*., 2007; Gregory, 2008). Other research reports strong evidence that CO_2 gas concentrations of 80% by volume applied for 70 sec, as required by law, are not sufficient to stun pigs adequately, as blood analyses conducted revealed very high concentrations of catecholamines after stunning (Hartung *et al*., 2002).

Clearly, the slaughtering process in swine abattoirs is one of the most stressful events that hogs experience in their lives. Thus, it is incumbent that a fast, optimized, pre-slaughter stunning method be used that prevents pain and suffering (Mota-Rojas *et al*., 2010b; Mota-Rojas *et al*., 2011a; Orozco-Gregorio *et al*., 2010; Mota-Rojas *et al*., 2012b). For this reason, the principal objective of this review is to characterize the physiological and biochemical events that occur in hogs during pre-slaughter stunning with CO_2, as they relate to animal welfare.

ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL AXIS AND BIOCHEMICAL CHANGES IN SWINE IN RESPONSE TO STRESS

Knowledge of the molecular bases of stress responses, aggressive behaviour and inter-individual variation in pigs is very limited (Murani *et al*., 2010). Stress responses promote the maintenance of homeostasis and adaptation to the physiological and psychosocial challenges of a changing environment; a complex process that involves the coordinated activation of behavioural, autonomic and neuroendocrine reactions. Concomitantly, pathways that promote vegetative functions such as growth, reproduction and feeding are inhibited, to a degree that depends on the duration and intensity of the stressor (the so-called biological cost of the stress response) (Moberg, 2000).

Aggression, meanwhile, is a powerful stressor that has been shown to activate the hypothalamic-pituitary-adrenocortical (HPA) axis, as well as the sympatho-adrenomedullary (SAM) system in various species, including swine (Fernandez *et al*., 1994; Otten *et al*., 1999; D'Eath *et al*., 2010). In pigs, aggression commonly occurs when unfamiliar individuals are mixed together, as this act upsets the existing order of social dominance (Meese and Ewbank, 1973). In addition to its negative effects on animal welfare, aggression has also been shown to have a negative impact on immune responses (Tuchscherer *et al*., 1998), growth performance (Sherritt *et al*., 1974) and product quality in pigs (Terlouw *et al*., 2008; D'Eath *et al*., 2010). In turn, aggression is affected by the functional properties of the HPA axis and the SAM system (Haller and Kruk, 1993). Baseline levels of glucocorticoids are inversely related to aggressiveness in various species, including pigs (Haller and Kruk, 1993; Tuchscherer *et al*., 1998; Touma *et al*., 2008).

CRH (corticotrophin-releasing hormone) and vasopressin act synergistically to activate the expression of proopiomelanocortin, the precursor of adrenocorticotropin (ACTH), in the pituitary gland by activating CRH type 1 and vasopressin V1B receptors, respectively. CRH is the dominant trigger of HPA axis activation during acute stress while vasopressin, itself a weak ACTH secretagogue, is important in mediating responses to chronic stress. Vasopressin also has an important function in controlling aggressive behaviour in various species (Caldwell *et al.*, 2008), including swine (D'Eath *et al.*, 2005). The CRH-binding protein functions as a buffer for CRH and related peptides and plays an inhibitory role in modulating CRH activity. ACTH stimulates the synthesis and secretion of glucocorticoids from the adrenal cortex via melanocortin type 2 receptors; i.e., glucocorticoids like cortisone, corticosterone (Randall *et al.*, 2002) and cortisol (Madej *et al.*, 2005). A general metabolic effect of cortisol favours the accretion of fat in blood circulation (Skrlep *et al.*, 2009). The action of glucocorticoids on target tissues is mediated by the glucocorticoid receptor, which in the hypothalamus, pituitary and hippocampus acts to end the stress response (Steckler, 2001). Though not involved directly in the action of the HPA axis, urocortins may play a modulatory role by stimulating the type 2 CRH receptor (Bale, 2005).

PHYSIOPATHOLOGY OF STUNNING WITH CO_2

Carbon dioxide is a colourless, odourless gas with a slightly acid taste that is present in all living organisms and is the principal molecule liberated when food is metabolized. The anaesthetic properties of CO_2 have been known for over 150 years. The presence of CO_2 in the blood stimulates respiration, heart rate and blood pressure, before it is eliminated through the lungs in expired air in a proportion of approximately 4%. The combined effects of these three factors facilitate the later exsanguination of the animals, which is why CO_2 is added to oxygen or normal air in traditional systems of anaesthesia using gas mixtures. The CO_2 anaesthesia system, one that is used more and more in large industrial slaughterhouses, consists in exposing animals to a mixture of gases rich in CO_2 (70-90%) to induce insensibility within 30-to-39 s and leave the animals completely stunned in barely 1 min (Gregory *et al.*, 1990; Alvarez-Alvarez, 2010).

When CO_2 is respired, it combines with water to form H_2CO_3, which produces high concentrations of H^+ ions in the pig that result in a state of acidosis at the cellular level (Guyton and Hall, 2001; Mathews *et al.*, 2006).

Once introduced into the organism, CO_2 produces insensibility, but leaves no undesirable chemical residues in the pork meat. In this procedure, hogs are placed in a chamber with an atmospheric concentration of CO_2 of 80 to 90% for a period sufficient to render them unconscious. Later they are killed by bleeding. The CO_2 stunning system does not require that animals be forcibly restrained and allows stunning in groups, which reduces stress. Stunning is achieved by depressing the neuronal function as a consequence of hypercapnic hypoxia and reducing the pH of the central nervous system (Velarde *et al.*, 2000).

When exposed to the gas (80-90%), hogs lose sensibility and consciousness by around 30 sec. In swine, the extent of the stunning effect can be interpreted easily by testing different reflexes and/or observing their behaviour; for example, using stimuli that induce pain (such as pricking the snout) or other clinical reflexes, including the corneal reflex, panting, the rhythm of the respiratory frequency, convulsions and struggling movements upon being hooked prior to disgorgement (Nowak *et al.*, 2007).

When blood pCO_2 (partial pressure of CO_2) levels increase, the pCO_2 in the interstitial liquid of the raquid bulb and the cerebrospinal liquid also rise. In both liquids, carbon dioxide reacts immediately and paradoxically, liberates more hydrogen ions inside the chemically-sensible respiratory zone when blood concentrations of carbon dioxide increase than when the concentration of hydrogen ions varies. This explains why changes in blood CO_2 levels produce such important variations in the activity of the respiratory centre (Guyton and Hall, 2001). Today, questions have been raised with respect to animal welfare, as inhaling high concentrations of CO_2 may cause distress before unconsciousness occurs. In particular, the bodily movements seen during the early part of CO_2-induced anaesthesia in swine have elicited questions related to stress (Martoft *et al.*, 2002), as they could be caused by physiopathological changes brought on by exposure to the gas, such as: Lactic academia, hyperglycaemia, hyperkalemia, hypercalcemia and respiratory and metabolic acidosis, seconds before the pigs enter the anaesthetised state (Becerril-Herrera *et al.*, 2009b). To confirm whether or not animals experience stress during induction to, and awakening from, CO_2 anaesthesia, a description of the rapidity and severity of the accompanying changes in CNS activity is needed (Martoft *et al.*, 2002). High concentrations of CO_2, typically around 90% in ambient air, are used to provide pre-slaughter anaesthesia in swine and poultry, as well as short-term surgical anaesthesia and euthanasia in laboratory animals. It is well-known that arterial PCO_2 changes rapidly and that neuronal suppression begins soon after. However, the speed and extent to which inhaling high concentrations of CO_2 overloads the buffer systems of brain tissue in comparison to arterial blood, thereby causing respiratory acidosis, has not yet been documented (Martoft *et al.*, 2003).

To determine the changes that modulate respiratory dynamics in the presence of CO_2 (Martoft *et al*., 2002) analyzed the behaviour of 14 hogs weighing 25-to-35 kg (55-77 lbs) during stunning with CO_2. The pigs were tranquil before the start of induction. They grunted slightly, but in all other respects appeared calm, even while being hoisted. A standard set of behavioural changes was seen in almost all the animals during induction, anaesthesia and awakening: Upon entering the gas chamber, the pigs seemed inquisitive as they moved their heads slightly while sniffing and licking their snouts; this was followed by snorting. Approximately 10 sec after immersion, they breathed very deeply and shortly thereafter began to hyperventilate with long, deep inhalations followed by exhalations in short forced bursts. The physiological response to this phenomenon is attributed to the excess of CO_2 or hydrogen ions in the blood, which fundamentally stimulates the respiratory centre itself and greatly augments the strength of the inspiratory and expiratory signals sent to the respiratory muscles (Guyton and Hall, 2001), thus increasing pulmonary ventilation (Randall *et al*., 2002). An extraordinary increase in blood pCO_2 initially causes stimulation, but is followed by a depression of the breathing rate that ultimately ends in death (Guyton and Hall, 2001).

At the same time, the mean Heart Rate (HR) increased from baseline values of 116±10 beats/min to 135±10 beats/min. HR did not change noticeably during the first 10 sec of immersion, but by the end of the induction time, had decreased to 10±10 beats/min (in 9 out of 12 trials the heart had stopped beating by that time). When the pigs were returned to ambient air, HR returned to initial values approximately 240 sec after the start of induction (Martoft *et al*., 2002). This behaviour reflected in changes in HR occurs because when blood O_2 concentrations fall arterial chemoreceptors are stimulated and cause peripheral vasoconstriction and a reduction in HR and cardiac output; thus blood flow to many other body tissues is reduced in order to maintain the flow to the brain, heart and certain endocrine organs (Randall *et al*., 2002).

STUNNING OF HOGS WITH CO_2: PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS

In veterinary practice, techniques have been implemented to reduce the pain that animals suffer during routine ante-mortem handling, as in the case of CO_2 anaesthetic used in castrating pigs. Kohler *et al*. (1998) designed a study based on a randomised clinical trial with piglets assigned to 7 groups (Table 1). Three groups were castrated, under halothane gas, CO_2 anaesthesia, or without anaesthesia of the four control groups that were not castrated, one received halothane, another CO_2 anaesthesia, one was placed in the mask induction system while breathing room air and the other was simply held in the typical castration position. This study used 70 male VLS x Duroc x Pietrain piglets aged 3-4 weeks, provided by a breeder (member of the Swiss Pig Health Service SGD) with a mean body weight of 7.07 kg (SD 1.52 kg, min 4.0 kg, max 10.6 kg). All piglets were alert and free of clinical signs of illness. The three gas exposure conditions were:

- A mixture of 80% CO_2/20% O_2 administered for 30 sec
- 5% halothane in oxygen delivered for 60 sec through a vaporizer with manual temperature control
- Pigs were placed in the mask induction system while breathing room air, without anaesthesia

During the experimental procedure, a mask induction system was developed that was suitable for eventual use by upwards. A lightweight rubber face mask, a pressure relief valve and a 2 L respiratory gas tank were connected to the narrow opening of the cone that faced downwards. A scale to estimate and classify the pain felt by the pigs was designed, consisting of a 3-point score, in which: [0] = no reaction, [1] = moderate reaction and [2] = violent reaction.

The results obtained in this study showed that the pigs exposed to the combination of CO_2/O_2 vocalized and struggled more than those exposed to halothane gas during the period of induction to anaesthesia. The combination of 80% CO_2/20% O_2 during 30 sec produced irritation and aversion, but this behaviour was reversed during castration. Kohler *et al*. (1998) reported that no animals in the CO_2 group showed reactions to the surgical intervention, while the moderate movement observed in two piglets was not related to castration. Under halothane anaesthesia, half of the group moved and showed some temporary defensive reaction to the surgery. The depth of the anaesthesia was judged insufficient, though castration could be performed easily with no violent defence. In contrast to these observations, the non-anaesthetized group, with only one exception, showed continuous violent struggling, vocalizations and a violent defence to castration.

Kohler *et al*. (1998) concluded that CO_2 anaesthesia was considerably stressful for the piglets, as judged by their struggling, vocalization and strenuous breathing during induction. Violent struggling and vocalization were, however, also observed during exposure to room air, while reactions to halothane anaesthesia were short-lived and, at most, moderate. In contrast, the non-anaesthetized control group showed a violent defensive reaction, as

Table 1: Experimental design from Kohler *et al.* (1998) on the anaesthetic effects of CO_2 and halothane gas in castrated and non-castrated piglets (Control group)

Castrated piglets	Non-castrated piglets (Control group)
Group 1: CO_2 anaesthesia (n = 10)	Group 4: CO_2 anaesthesia (n = 10)
Group 2: Halothane anaesthesia (n = 10)	Group 5: Halothane anaesthesia (n = 10)
Group 3: Without anaesthesia (n = 10)	Group 6: Placed in the mask induction system while breathing room air (n = 9)
	Group 7: Held in castration position only (n = 11)

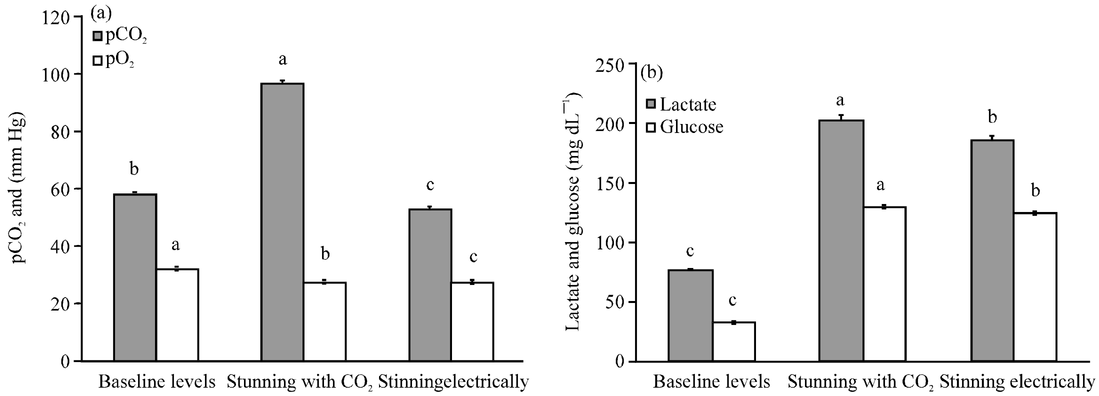
assessed by their continuous struggling and vocalization, with the exception of one piglet. Although, CO_2 induces profound surgical analgesia, it does not seem reasonable to advocate an anaesthetic regimen for castrating piglets that turns out to be more stressful for the animals than castration without anaesthesia.

Several reports dealing with animal welfare use blood indicators to describe the degree of stress and suffering in animals. In this vein, Nowak *et al.* (2007) reported that hogs stunned with different concentrations of CO_2 for different times, presented high concentrations of lactate and catecholamines. They found that applying an 80% concentration of CO_2 for 70 sec (GC8-70) generated levels of blood lactate of 8.8±6.84 mmol L^{-1}, the highest concentration in their study and showed significant differences ($p<0.01$) compared to the other groups that received higher concentrations with longer exposure times. In their study, Nowak *et al.* (2007) found no significant differences in the levels of the two catecholamines among the different CO_2 stunning procedures, though clinical parameters such as nose-picking (p = 0.002), heart activity after hoisting and gasping motions (p = 0.0001), indicated differences in levels of consciousness. This seems to indicate that the stunning method (gas, electricity) has a more significant influence on the levels of these substances than does the stress experienced shortly before slaughter. The GC8-70 procedure led to the highest median and overall lactate values (up to 35.8 mmol L^{-1}) and presumably, the greatest stress for the animals. Finally, these results indicate that swine experience the greatest stress after 80% CO_2 stunning, especially with 70 sec of exposure in the stunning chamber and after only 70 sec with 90% CO_2.

Recently, blood indicators of stress have been used with greater frequency to study stunning and anaesthesia with CO_2 in swine. Gerritzen *et al.* (2008) exposed 25 pigs to CO_2 (70% CO_2 + 30% O_2) before performing surgery (castration). Induction to analgesia was carried out in a transparent chamber. Only one piglet was placed inside at a time, where it was exposed to gas for 120 sec. Also measured were changes in blood parameters (pH, pCO_2, pO_2, SO_2c, EB, glucose and lactate) due to exposure to the gas. Two blood samples were taken from the jugular vein of each piglet: 1) before exposure to the gas and 2) after castration. According to these results, significant differences ($p<0.001$) were found before and after exposure to CO_2, differences that were reflected in pH (7.4±0.02 vs. 7.1±0.02), pCO_2 (5.7±0.27 vs. 8.2±0.46 kPa), and pO_2 (4.2±0.41 vs. 7.3±0.91). SO_2c showed no significant differences. With respect to EB values, glucose and lactate also showed significant differences ($p<0.001$) when the before and after values of exposure to carbon dioxide were compared, as follows: EB: -0.2±0.5 vs. -9.4±0.8 mmol L^{-1}; glucose: 7.2±0.16 vs. 9.9±0.38 mmol L^{-1} and lactate: 7.5±0.5 vs. 13.9±0.8 mmol L^{-1}.

Changes in blood pH affect enzyme reactions related to energy production and spur changes in membrane permeability and electrolyte management. It is hypothesised that this pH point is already too low to regenerate physiological equilibrium. Thus, it can be concluded that both the CO_2 concentration in the gas mixture and exposure time are critical factors in the successful induction of unconsciousness, on the one hand and on the other, the risk of death in young piglets. The induction and maintenance of insensibility and unconsciousness during castration of piglets is possible using 70% CO_2 with 30% O_2; though it cannot be denied that inhaling and inducing anaesthesia with CO_2 causes discomfort or stress. However, un-anaesthetised castration or local inter-testicular anaesthesia was assumed to cause more stress and pain than the induction of general CO_2 anaesthesia. It is clear that CO_2 anaesthesia induces an analgetic state for only a short time; hence, the use of additional analgesic drugs is recommended (Gerritzen *et al.*, 2008).

Some studies now focus on a direct relationship between exposure time to CO_2 in pigs, animal welfare and blood indicators of stress and suffering in commercial slaughterhouses in Mexico. Becerril-Herrera *et al.* (2009a) reported that hogs should be exposed to 80% CO_2 quickly and for a period of just 60 sec. The objective of their study was to compare the effect of the electric and CO_2 stunning methods on energy profiles, acid imbalances and gas exchanges, as a means of determining animal welfare. Figure 1a and b show the mean and standard error of the indicators of the energy profile, acid imbalance and blood gas of the pigs stunned before slaughter using these two methods together with the reference values that determine the effect that stunning had on the physiology of the animals and therefore, their welfare. Results indicate significant differences ($p<0.05$) between treatments for all variables from animals stunned with CO_2, as those pigs



No. of pigs	159	247	252
pH:	7.43±0.006^{c}	6.93±0.006^{c}	7.14±0.007^{b}

No. of pigs	159	247	252
Haematocrit (%)	30.30±0.37^{c}	51.67±0.38^{c}	4.4±0.32^{b}

Fig. 1a-b: Mean and standard error of energy metabolism, acid-base balance and blood gases from stunned swine. (Becerril-Herrera *et al*., 2009a), ^{a,b,c} pCO$_2$, pO$_2$, haematocrit, glucose, lactate and pH levels show statistically significant differences according to the Tukey test ($p<0.05$)

showed hypercapnia (96.3±90.93 mmHg), hyperglycaemia (201.49±4.41 mg dL^{-1}), lactic academia (129.49±0.57 mg dL^{-1} or 9.85±0.06 mmol L^{-1}) and an increase in haematocrit (51.67±0.38%). In the case of electrical stunning, the pigs showed a decrease in blood pH, pCO$_2$ and pO$_2$ (Fig. 1a). Hyperglycaemia and lactic acidemia indicate stress before sacrifice. There was also evidence of dehydration in all the stunned animals, measured as an increase in haematocrit; a finding that may be indicative of poor management during lairage Fig. 1b. Therefore, both of these conditions may affect animal welfare.

Though animals are not physically restrained when stunned in the CO_2 chamber, this method produced a higher percentage of altered indicators compared to baseline levels. In the chamber, stunning is achieved through a neuronal function caused by hypercapnic hypoxia and diminishing pH in the central nervous system (Velarde *et al*., 2000). In addition, stunning in the CO_2 chamber increases the anaerobic oxidative metabolism that increases glucose levels in the blood stream and triggers the intracellular flow of K^+ ions due to hydrogen ions, causing metabolic acidosis.

According to Pollard *et al*. (2002), glucose levels are an indicator of stress and glycaemia is subject to hormonal control. Therefore, the glucagon, glucocorticoids, adrenalin, thyroid hormones, growth hormones and progesterone become hyperglycaemic and trigger gluconeogenesis and glycogenolysis, suggesting the use of glucose by the tissues (Kaneko, 1997). Lactate is a metabolite produced through muscular glycogenolysis due to a lack of glucose phosphatase 6, which is necessary for glycogen synthesis. The lactate that forms in the muscles is transported by the blood to the liver, where it is transformed into glucose. Another source of lactate is anaerobic glycolysis: in the absence of oxygen, pyruvate is reduced to lactate through lactate dehydrogenase (Kaneko, 1997). The increase in haematocrit is attributed to contractions of the spleen, in part due to diminished plasma volume. Those contractions are an effect of the catecholamines liberated during sympathetic stimulation (Jain, 1993) and provide a large quantity of oxygenated erythrocytes to the muscular mass that allows the animal to become more active. From the animal welfare point of view, Velarde *et al*. (2000) considered that CO_2 stunning was less efficient than electronarcosis (head and side methods), since all physiological reflexes tested, for example the corneal reflex and sensibility to pain, with the exception of spontaneous respiration, were clearly superior to those found with electric stunning.

CO_2 stunning caused lactic academia, hyperglycaemia, hyperkalemia, hypercalcemia and respiratory and metabolic acidosis in swine, seconds before they entered the anaesthetised state. On the other hand, electrocution triggered hypernatremia and hyperglycaemia; therefore, CO_2 stunning caused more severe alterations and hence, may have compromised animal welfare more, compared to electrical stunning (Becerril-Herrera *et al*., 2009b).

Turning to another study, in their examination of blood indicators of stress and animal suffering, Edwards *et al*. (2010a, b) found that increases in blood lactate concentrations associated with pre-slaughter stresses, such as aggressive handling immediately prior to stunning, have been shown to exercise detrimental effects on pork quality. Edwards *et al.* (2010a) used 128 cross-bred barrows that were allowed to rest at the slaughterhouse for 8 h. Following this lairage period, the pigs were stunned with a CO_2 system. In their results, upon exsanguination blood lactate levels ranged from 4 to 19.7 mM; though the authors do not mention the degree of stress and aversion shown by the hogs due to the effect of CO_2, the blood lactate concentrations presented in their results show higher levels than those reported by Nowak *et al.* (2007), Gerritzen *et al.* (2008) and Becerril-Herrera *et al.*, (2009b). This suggests that those pigs may have manifested a high degree of aversion and experienced greater pain during stunning, thus dramatically reducing their welfare.

Warriss *et al.* (1994) showed that exsanguination blood lactate concentrations were predictive of differences in the stress levels evaluated subjectively at different slaughter facilities, but were unable to relate the stress experienced by an individual pig at slaughter to the subsequent quality of its meat. Edwards *et al.* (2010a), however, did succeed in demonstrating a relationship between blood lactate concentrations of individual pigs at slaughter and their subsequent meat quality characteristics, as exsanguination blood lactate concentrations correlated positively ($r = 0.22$, $p = 0.02$) with drip loss, but negatively with 60 min pH ($r = 0.32$, $p = 0.0004$). Although, the r-values observed in this experiment are relatively low, they suggest that under conditions of low-stress pre-slaughter handling, exsanguination blood lactate is a potential predictor of the rate of post-mortem metabolism; i.e., high blood lactate predicts a more rapid drop in early post-mortem pH, which in turn results in greater drip loss. These data seem to suggest that there is a relation among aversion and suffering during stunning with CO_2, animal welfare and reductions in the quality of the pork meat obtained due to dripping loss.

To counteract this stressful phenomenon during the pre-slaughter stunning of swine, it has been suggested that use of the CO_2 method of stunning the animals in abattoirs be re-evaluated and rethought, so as to assure their welfare. Such reassessments seem to favour reducing CO_2 concentrations and using mixtures that include other, more noble, gases that are less aggressive. In a recent study of this problem, Dalmau *et al.* (2010) argued in favour of using other gases, such as argon. The objective of their study was to ascertain whether 90% argon (AR) by volume in atmospheric air and gas mixtures of 70% N_2 with 30% CO_2 (70N30C) and 85% N_2 with 15% CO_2 (85N15C) by volume in atmospheric air used in a commercial diplift stunning system generate aversion in slaughter-weight pigs, through aversion-learning tests and observations of the pigs' behaviour in the chamber upon exposure to the different mixtures.

In that study, 50% of the pigs exhibited muscle jerking; of those animals, 41% showed jerking before loss of posture, 39% at the time of loss of posture, and 20% after loss of posture. Following Raj and Gregory (1996), muscle jerking before, or at the same time as, the loss of posture is associated with conscious animals performing escape attempts. On the other hand, muscle jerks after loss of posture are associated with involuntary convulsions in unconscious animals (Forslid, 1987, 1992). Rodriguez *et al.* (2008), however, stated that animals could be conscious during these movements, regardless of the loss of posture.

Therefore, Dalmau *et al.* (2010) reported that such muscle jerks should be studied regardless of the loss of posture, and not be considered as voluntary escape attempts or involuntary convulsions, but only as muscular excitation that occurs before, at the time of, or after, loss of posture. Raj (1999) described that muscle jerking takes longer when pigs were exposed to argon or a mixture of argon and CO_2 than under exposure to a high CO_2 concentration. In their study, the presence of jerking (percentage of animals that showed convulsions and their intensity) was higher with mixtures of CO_2 and N_2 than when argon was used. As these muscle jerks usually occurred at the same time as, or just after, loss of posture (59%), their results concord with those obtained from analyses of other indicators, where it was observed that 90% AR required more time to induce unconsciousness than did mixtures of N_2 and CO_2.

Currently, pigs are usually stunned with high CO_2 concentrations but, as these conditions can cause aversion, gas mixtures with lower CO_2 concentrations, such as 70 N_2/30 CO_2 and 85 N_2/15 CO_2, or high concentrations of inert gases, such as argon, could constitute viable alternatives. However, as CO_2 concentrations decrease, less aversion to exposure is seen; therefore, from an animal welfare point of view, 90% of argon by volume or the lowest possible CO_2 concentration required to effectively stun the pigs are recommended (Dalmau *et al.*, 2010). Indeed, argon is recommended as a welfare-friendly alternative to carbon dioxide for stunning/killing both pigs and poultry (FAWC, 2003; Raj, 2006). Stunning or killing with inert gases, especially argon, has been studied extensively in farm animals, including birds, as they have no intrapulmonary chemoreceptors capable of detecting inert gases and hence, show no aversion when initially exposed to hypoxia/anoxia induced by nitrogen, argon or mixtures of the two (Raj, 2006).

CONCLUSION

In the organism, CO_2 produces insensibility without leaving any undesirable chemical residues in the pork meat. Moreover, animals do not need to be restrained and can be stunned in group, which reduces stress levels. In this method, stunning is achieved by depressing the neuronal function as a result of hypercapnic hypoxia and a reduction of the pH of the central nervous system. CO_2 stimulates the respiratory centre, thus increasing markedly the strength of the inspiratory and expiratory signals sent to the respiratory muscles and increasing pulmonary ventilation. Also, it has been argued that exposure to 90% CO_2 causes less physical stress than the use of an 80% concentration, as it results in a less pronounced metabolic acidosis. On the other hand, the 90% concentration can cause behavioural aversion. In fact, the mixture of 80% CO_2/20% O_2 produces a larger number of vocalizations and struggling when compared to young hogs that were exposed to halothane gas during the induction period to anaesthesia. In addition, it has been demonstrated that pigs exposed to a concentration of 80% CO_2 for 70 sec have higher blood lactate concentrations. Blood analyses revealed very high concentrations of catecholamines, as well as lactic academia, hyperglycaemia, hyperkalemia, hypercalcemia and respiratory and metabolic acidosis in swine, seconds before entering the anaesthetised state when CO_2 at 80% was applied for 60 sec. Blood indicators of stress, such as lactate, have been used with increasing frequency to study the stunning process and anaesthesia with CO_2 in swine. To counteract the phenomenon of aversion to CO_2, studies have shown that 90% AR is recommendable as a welfare-friendly alternative to carbon dioxide for stunning/killing pigs and poultry. It is clear that animal welfare is not taken into account when stunning with CO_2 is used, which makes it an even more questionable method. Finally, animal welfare is a requirement for trade with the European market, so meat products that satisfy this exigency through good handling practices have added value and enjoy purchaser preference.

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