Meat Science 81 (2009) 233–237

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

CO_2 stunning may compromise swine welfare compared with electrical stunning

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ARTICLE INFO

Article history:
Received 9 April 2008
Received in revised form 24 July 2008
Accepted 28 July 2008

Keywords:
Animal welfare
Carbon dioxide electrical
Stunning
Electronarcosis
Pigs

ABSTRACT

The effects of two different stunning methods on critical blood values in fattening pigs at a federal inspection slaughtering plant were monitored. A total of 658 pigs from the same genetic line and origin, were randomly assigned to 3 treatments: reference baseline levels (resting pigs; T1), stunning with CO_2 (T2) and stunned electrically (T3). Energetic profile, acid imbalance and blood gas levels, were monitored. Significant differences ($p \leqslant 0.05$) between treatments for all variables were found, CO_2 stunned pigs showed hypercapnia, hypercalcemia, hyperglucemia, lactic acidemia, and an increase in haematocrit, coupled with reduced pH, P_{O_2}, and Na; electrically stunned pigs had reduced blood pH, P_{CO_2} and P_{O_2}. The remaining indicators were increased in relation to the resting swine. Thus CO_2 stunning leads to a major imbalance because of mineral and acid base gaseous interchange, compared to electric stunning, thus possibly compromising animal welfare.

1. Introduction

Maintaining high standards of animal welfare during transportation and slaughter requires the appropriate equipment and supervision of employees. Besides, animals should be unconscious at the time of slaughter in order to avoid pain and stress during the procedure (Gracey, 1989; Grandin, 2003). Most developed countries and many developing countries have laws that require stunning before sacrifice (FAO, 2001). Stunning is based on producing insensibility by striking the animal or other means.

Sacrifice of swine is carried out by bleeding the arteries and veins of the brachiocephalic trunk that interrupts the nutrient and oxygen supply to the brain, causing death of the animal. Therefore acceptable stunning systems must guarantee quick action rendering the animal unconscious without pain, and the unconscious state must be prolonged till the animal's death (Quiroga & García, 1994).

A stunning system can be reversible or irreversible. In the first case, animals can recover consciousness before death; therefore, the time between stunning and bleeding is a determining factor with regard to stunning efficiency. On the contrary, irreversible stunning systems "stun" and cause death of the animal simultaneously. In this case, the objective of the sacrifice is to drain the blood from the carcass, for which time would not be critical from the animal welfare point of view (Quiroga & García, 1994; Velarde, Faucitano, Manteca, & Diestre, 2000a).

Currently, the most frequently used methods for stunning swine are electric stunning and exposure to carbon dioxide. The aim of this study was to compare the effect of these stunning methods on the energetic profile, acid imbalances, as well as gaseous interchange, as means to determine animal welfare.

2. Material and methods

2.1. Location

This study was carried out in a federal inspection plant in Central Mexico.

2.2. Experimental handling

A total of 658 Mexican swine from a cross of Yorkshire–Landrace mother and a Pietrain sire, were monitored. The day before they left the farm for transportation to the slaughterhouse, random blood samples were taken from 159 pigs approximately 155 days old. This sample was taken as both baseline and reference sample. The next day the animals were transported for 7.5 h from the farm to the slaughterhouse and records were kept until they were sacrificed.

Transportation was done according to the animal care regulations in Mexico (official Mexican regulation NOM-024-ZOO-1995).

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0309-1740/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.meatsci.2008.07.025

2.3. Treatment distribution

The stunning was distributed randomly considering the treatments from Table 1.

2.4. Energetic profile, acid imbalance and blood gas tests

After pigs had been rested for 4 h in pens, stunning and bleeding were carried out and immediately after hanging and bleeding of major blood vessels, a single 1 mL blood sample was obtained from the jugular vein by anterior neck puncture using a syringe containing lithium heparin. The blood sampling took approximately 20–30 sec. Haematocrit (%), glucose (mg/dL), serum electrolytes [Na^+, K^+ and Ca^{2+} (mmol/L)], blood lactate (mg/dL), partial pressure of carbon dioxide [P_{CO_2} (mm Hg)] and oxygen [P_{O_2} (mm Hg)] levels, were obtained by means of an automatic blood gas and electrolyte analyzer (GEM Premier 3000, Instrumentation Laboratory Diagnostics S.A. de C.V. Mexico). In addition, the time interval between stun to stick was monitored.

2.5. Stunning method

Pigs were stunned before sacrifice by the following methods: electrically (head only stunning) using a restrainer at a current over 250 mA with a voltage of 400 V, for 2 sec and by introducing them into a CO_2 chamber with 70% CO_2 atmosphere for approximately 60 sec, in a one-gondola dip-lift system.

2.6. Statistical analysis

The results obtained were analyzed through a completely random design, which model was

$$Y_{ij} - \mu + \iota_i + \xi_{ij}$$

$$i = 1, 2 \ldots \text{treatment}$$

$$j = 1, 2, 3 \ldots \text{repetitions}$$

where: Y_{ij} = response variable; μ = general mean; ι_i = treatment effect; ξ_{ij} = random error.

The results were analyzed according to the proposed model and through the following procedures:

Time from stunning to bleeding was analyzed with an independent sample *t* test for independent variables.

In order to determine statistical differences in the treatments the variables were evaluated using the Tukey test ($p < 0.05$).

The pH variable was statistically analyzed using the Kruskal–Wallis test.

Linear regression analysis of each of the stunning methods: regarding the animals anaesthetized with CO_2, P_{CO_2} was used as a dependent variable, and P_{O_2}, glucose, haematocrit, lactate and pH as independent variables. A similar process was carried out for the electrically stunned animals.

The SAS Institute (1997) computer program was used.

3. Results

Table 2 shows the mean and standard error of the mean for the interval between stunning and bleeding, with high significant differences between treatments ($p < 0.001$), CO_2 stunned pigs having a longer interval to sticking compared to electrically stunned hogs.

Table 1
Treatment distribution according to stunning method

Treatment 1	159 resting pigs were used as reference values
Treatment 2	247 pigs were stunned with CO_2
Treatment 3	252 pigs were stunned electrically

Table 2
Mean and standard mean of the time interval between stun and bleeding

Variable	Stunning with CO_2 $n = 247$	Stunning electrically $n = 252$	*P*
	Mean ± SEM	Mean ± SEM	
Time interval between stun and stick (seconds)	92.62 ± 1.75	63.51 ± 1.42	0.0001

n, number of observed pigs; SEM, standard error of the mean.

Table 3 shows the mean and standard error of the energetic profile indicator, acid imbalances and blood gas of the pigs stunned by two different methods before sacrifice. It also shows the reference values which determine the effect stunning had on the physiology of the animal and therefore its welfare. Significant differences ($p < 0.05$) between treatments for all variables were found. Results indicate significant differences ($p < 0.05$) between treatments for all variables from animals stunned with CO_2, these pigs showed hypercapnia, hypercalcemia, hyperglucemia, lactic acidemia, and an increase in haematocrit, coupled with reduced pH, P_{O_2}, and N. In the case of electrical stunning, pigs showed a decrease in blood pH P_{CO_2} and P_{O_2}.

Swine stunned with CO_2 had increased P_{CO_2}, potassium, calcium, glucose, lactate and haematocrit, coupled with a decrease in pH, P_{O_2} and sodium. The electric stunning group showed decreased blood pH, P_{CO_2} and P_{O_2}; the remaining indicators increased in relation to the baseline values. The nonparametric statistical analysis applied to the pH variable showed significantly different results ($p < 0.01$) between stunning methods and baseline levels.

Nevertheless, it is worth mentioning that both stunning methods caused hyperglucemia and lactic acidemia, indicating stress before sacrifice. There was also evidence of dehydration in all the stunned animals, measured as a haematocrit increase, which may indicate poor management during lairage; both conditions may affect animal welfare.

Linear regression analysis for animals stunned with CO_2 (Table 4), indicate that P_{O_2}, glucose and pH, negatively correlated with P_{CO_2}, this means that when P_{CO_2} increases in the blood, oxygen, glucose and pH are diminished. In addition positive correlations between P_{CO_2} and haematocrit percentages, as well as lactate

Table 3
Mean and standard error of energetic metabolism, acid–base balance and blood gases from stunned swine

Variables	Baseline levels $n = 159$	Stunning with CO_2 $n = 247$	Stunning electrically $n = 252$
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Blood pH*	7.43 ± 0.006^a	6.93 ± 0.006^c	7.14 ± 0.007^b
P_{CO_2} (mm Hg)	58.03 ± 0.42^b	96.39 ± 0.93^a	53.04 ± 0.63^c
P_{O_2} (mm Hg)	32.02 ± 0.57^a	27.55 ± 0.58^b	27.48 ± 0.52^b
Na^{2+} (mmol/L)	141.57 ± 0.17^b	140.64 ± 0.67^b	146.13 ± 0.47^a
K^+ (mmol/L)	5.40 ± 0.03^c	14.20 ± 0.14^a	9.91 ± 0.13^b
Ca^{++} (mmol/L)	1.27 ± 0.006^c	1.45 ± 0.006^a	1.29 ± 0.005^b
Glucose (mg/dL)	76.57 ± 0.44^c	201.49 ± 4.41^a	184.98 ± 3.55^b
Lactate (mg/dL)	33.10 ± 0.45^c	129.49 ± 0.57^a	124.67 ± 0.87^b
Haematocrit (%)	30.30 ± 0.37^c	51.67 ± 0.38^a	44.35 ± 0.32^b

[a,b,c] In the same row are statistically different, Tukey ($P \leqslant 0.05$).
n, number of observed pigs; SEM, standard error of the mean.

Table 4
Significant correlated variables in 247 pigs stunned by CO_2

Dependent variable (y)	Independent variable (x)	Linear equation ($y = b + mx$)		R	F & P values
		b ± SEM	m ± SEM		
P_{CO_2} (mm Hg)	P_{O_2} (mm Hg)	122.14 ± 2.41	−0.93 ± 0.08	−0.59	127.80; 0.0001
	Glucose (mg/dL)	119.99 ± 2.34	−0.11 ± 0.01	−0.57	113.84; 0.0001
	Haematocrit (%)	26.04 ± 6.66	1.35 ± 0.12	0.56	112.80; 0.0001
	Lactate (mg/dL)	19.84 ± 12.31	0.59 ± 0.09	0.37	38.80; 0.0001
	pH	426.53 ± 54.83	−47.57 ± 7.90	−0.36	36.26; 0.0001

The independent variables were numbered according to the correlation coefficient (R. value). The F and P values observed in the table come from the variance analysis in the linear regression analysis. Parameters b and m in the linear equation are observed with their corresponding standard error mean (SEM).

Table 5
Significant correlation variables in 252 pigs stunned electrically

Dependent variable (y)	Independent variable (x)	Linear equation ($y = b + mx$)		R	F & P values
		b ± SEM	m ± SEM		
P_{CO_2} (mm Hg)	K^{++} (mmol/L)	39.55 ± 3.09	1.35 ± 0.30	0.27	19.59; 0.0001
	pH	217.37 ± 38.78	−23.003 ± 5.43	−0.26	17.94; 0.0001
	Glucose (mg/dL)	44.81 ± ± 2.12	0.04 ± 0.01	0.24	16.36; 0.0001
	Ca^{++} (mmol/L)	16.49 ± 9.57	28.19 ± 7.35	0.23	14.69; 0.0002
	P_{O_2} (mm Hg)	59.28 ± 2.18	−0.22 ± 0.07	−0.18	9.001; 0.0030

The independent variables were numbered according to the correlation coefficient (R value). The F and P values observed in the table come from the variance analysis of the lineal regression analysis. Parameters b and m in the lineal equation were observed with their corresponding standard mean error (SME).

concentrations were found. All correlations were statistically significant.

Table 5 shows the linear regression analysis from animals stunned electrically, positive correlations were found for P_{CO_2} with potassium, glucose and calcium levels; this means that while P_{CO_2} increases, the central respiratory nervous system is affected as a consequence, and potassium, calcium and glucose have major mobility in the blood stream. On the other hand, negative correlations were found in pH and P_{O_2} with the dependent variable (P_{CO_2}).

4. Discussion

Shaw and Turne (1992), and Hartung, von Müffling, and Nowak (2008), reported that after sacrifice most stunning methods lead to an increase in plasma catecholamines, cortisol, endorphins, lactate, glucose, calcium, magnesium, and proteins among others; there can be major alterations in constituents without apparently compromising animal welfare. This study shows the physical-metabolic effects which are triggered when swine are stunned by two different methods.

4.1. Interval between stun-stick

Anil and McKinstry (1998) recommend that when head only electrical stunning is used with low voltage, pigs should be bled within 15 sec to avoid animal welfare and carcass quality concerns. However, when head to side of body stunning is used with high voltage, bleeding times may be up to 180 sec (Moreno, 2006). In the present study there was a mean interval of 63.5 sec for the electrical stunning group. It is well known that electrical stunning of animals is a reversible and recoverable state; most researchers agree that pigs should be bled within 30 sec to prevent them re gaining consciousness.

Velarde, Gispert, Faucitano, Manteca, & Diestre 2000b and Moreno (2006) indicate that CO_2 stunned pigs recover within 1–3 min after withdrawal from the chamber, whereas they die if they are left breathing the CO_2 for 4–5 min. Therefore, time of exposure is important as well as the interval between stun and stick, which should be within 60 sec, according to some European regulations (Atkinson & Algers, 2007). Current research by Hartung et al. (2008), shows that exposure of pigs to a CO_2 atmosphere of 80% volume for 70 sec is not sufficient for a proper stun. In the present study the average time taken from the CO_2 gondola to hanging and bleeding was quite high (92.6 sec). Atkinson and Algers (2007) showed that only 30% of all pigs in 7 abattoirs studied, where stuck within 60 sec. There are difficulties for many abattoirs to achieve the 60 sec due to technical design of the gondolas and the shackle line. In this study, pigs were stunned in gondolas with capacity for 4 animals at a time.

4.2. CO_2 stunning

When the animals are stunned in the CO_2 chamber, they are not physically restrained; there were a larger percentage of altered indicators in comparison to the baseline levels. Stunning is achieved through a neuronal function caused by hypercapnic hypoxia and diminishing pH in the central nervous system (Velarde et al., 2000a). In addition, stunning in the CO_2 chamber increases the anaerobic oxidative metabolism that increases glucose levels in the blood stream and triggers intracellular flow of K^+ ions by hydrogen ions, causing metabolic acidosis. Exposure to CO_2 stimulates the respiratory rate and can lead to respiratory distress (Raj & Gregory, 1995).

Considering the inverse proportional relation in the partial pressure of the blood gas (P_{CO_2}, P_{O_2}), Haumann (1989) reports that swine become unconscious with a mixture of 80% CO_2 and 20% air, which proves that the carbon dioxide produces the anesthesia and this is not caused by lack of oxygen (gasping), since experimental evidence after monitoring the oxygen content in the blood (Ring, 1988) and evaluating the recovery time of the animals (Laursen, 1983), suggests that gasping for air does not play an important role in CO_2 stunning.

Introducing animals in unfamiliar places such as gas chambers or the entrance to the slaughterhouse can cause anxiety or stress. Raj and Gregory (1995) found that swine exposed to CO_2 were more reluctant to enter a corral to eat apples, than swine that had been exposed to argon. Hartung, Nowak, Waldmann, and Ellerbrock (2002) observed that 80% CO_2 was not enough to eliminate all reflexes after 70 sec of exposure in swine. Gregory, Mohan Raj, Audsley, and Daly (1990) claim carbon dioxide is unpleasant to breath due to its acid aroma, which can be uncomfortable at high concentrations, causing the sensation of asphyxia. Even though this phenomenon only affects the animals in the first stages of anesthesia, Gregory (1994) considers that it is enough for it to be classified as an undesirable and inhumane stunning system.

Zeller, Schatzmann, and Imhof (1987) studied the convulsions swine suffer when stunned with CO_2, and suggest that part of the convulsive episodes happen in the relaxation phase, pigs remaining conscious, because of which they doubt that this is a humanitarian stunning system. Similarly, Prändl, Fischer, Schmidhofer, and Sinell (1994) proved through electroencephalographic studies, that animals stunned with CO_2 lost consciousness only after prolonged excitation and exposure to the gas for 40 sec, which from an animal welfare point of view, is considered inadequate since the animals reaction caused a high state of excitation, that translates into greater stress and renders poor quality meat. On the other hand, Forslid (1982) studied the electroencephalograms of swine anesthetized with CO_2 and found that the convulsive attacks happened after loss of consciousness.

These anxiety attacks trigger catecholamine release, which in turn causes an increase in cardiac rate, oxygen consumption and body temperature; diminishes pH, causes accumulation of lactic acid (Hambrecht, Eissen, & Verstegen, 2003; Hambrecht et al., 2004), increases gluconeogenesis and has a biphasic effect on potassium serum concentration. Initially, this causes a transitory increase in the level of potassium through stimulation of the α-adrenergic receptor, followed by hypokalemia due to β_2 receptor stimulation. This plays an important role in the development of fatigue due to exercise (Bia & DeFronzo, 1981); high levels of potassium serum have also been related to a stress response (Kock, Clark, Franti, Jessup, & Wehaunsen, 1987; Peinado, Fernández-Arias, Zabala, & Palomeque, 1993).

According to Pollard et al. (2002), glucose levels are an indicator for stress; glycaemia is subject to hormonal control. Therefore the glucagon, glucocorticoids, adrenalin, thyroid hormones, growth hormones and progesterone are hyperglycemic and activate gluconeogenesis and glycogenolysis, or infer the use of glucose by the tissues (Kaneko, 1997). There are a number of studies that describe the increase of serum or plasmatic levels in glucose as a consequence of stress (Bush, Smith, & Custer, 1981; Carrengher, Ingram, & Matthews, 1997; Franzman & Thorne, 1970; Hartmann, 1988; Hatting, Pitts, & Ganhao, 1988; Kocan et al., 1981; Kock et al., 1987; Seal, Verme, Ozoga, & Erickson, 1972). In response to stress, glucose levels increase due to catecholamine and glucocorticoids secretion.

Lactate is a metabolite originating in muscular glycogenolysis due to lack of glucose phosphatase 6, necessary for glycogen synthesis. Lactate forming in the muscle 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 by lactate dehydrogenase (Kaneko, 1997).

Haematocrit increase is attributed to spleen contractions, in part due to diminished plasma volume. The spleen contraction is the effect of liberated catecholamine during sympathetic stimulation (Jain, 1993). The spleen contraction provides a large quantity of oxygenated erythrocytes to the muscular mass that permits major activity for the animal.

4.3. Electrical stunning

Critical blood values from the electrically stunned animals showed metabolic acidosis, hypocapnia, hyperphosphotemia, hyperglycemia, lactic acidosis, and an increase in haematocrit percentage. As before (in the case of the animals stunned in the CO_2 chamber) the effect is caused by *ante mortem* handling, coupled to the elevated levels of serum sodium due to an excessive loss of water or reduced water ingestion (Bush, 1993).

Electronarcosis is a popular form of stunning in the swine sector, while at the same time is less recommended as compared to gas inhalation due to the fact that electrical stunning can produce a poorer quality final product and augment PSE carcasses (Barton-Gade, 1984; Velarde, Gispert, & Diestre, 1999). Although stunning eliminates the stress of bleeding, it induces physiological changes which can negatively affect the quality of the final product. These changes are due mainly to the increase in blood pressure, muscular activity and increased liquid exudation, also caused by a major denaturation of muscular proteins, with subsequent loss of meat quality caused by contusions, hemorrhages or fractures, or quality loss because of biochemical alterations responsible for muscular transformation of the meat. Indeed, electrical stunning causes higher incidences of PSE meat compared to the CO_2 stunning (Velarde et al., 2000b; Velarde et al., 2001), due to nervous system stimulation that accelerates *rigor mortis* and diminishes muscular pH while the musculature is still warm.

In addition, poor use of this technique results in lower quality carcasses with greater incidence of bruising, hemorrhages and bone fractures (Gregory, Moss, & Leeson, 1987; Larsen, 1982; Troeger, 1996). However, other authors point out additional influential factors such as predisposition to stress, high muscular development, excessive stress before sacrifice, muscular fiber lesions, immobilization, bleeding, and errors committed by personnel, as major causes of these defects (Lambooij, Merkus, & Hulsegge, 1992; Wenzlawowicz, 1996).

Larsen (1982) with electrically stunned animals found between 10% and 19% incidences of PSE meat, whereas animals stunned with CO_2 had an incidence between 2% to 9%. Channon, Payne, and Warner (2003) found that modifications in the amperage levels (0.9, 1.3, 2), contact time and type of electric stunning, had an effect on the incidence of PSE meat, hemorrhages, pH, exudation loss and fractures; they were higher than swine stunned in CO_2 chambers. Moreover, Channon, Payne, and Warner (2002) concluded that the use of electrodes on the head/side tends to decrease pH after sacrifice and produced pale meat with higher losses to exudation.

When comparing the two stunning methods, Mihajlovic, Turubatovic, and Radovanov (1993) observed that the dazed animals in the CO_2 chambers were less stressed moments before sacrifice, avoiding grunting sounds from the animals, while improving handling, and reducing PSE meat (2–6%), and blood splash that appears in the *Longissimus dorsi* muscle and bone fractures. Barton-Gade (1984) observed that electrically stunned swine went into *rigor mortis* earlier and there was more PSE meat 45 min *post mortem*, compared to CO_2 narcosis.

Velarde et al. (1999) did a comparative study in 4 slaughterhouses with electric and CO_2 stunning systems and the relationship to meat and carcass quality at 2 and 7 h *post mortem*; they found that the PSE incidence at slaughterhouses equipped with CO_2 inhalation systems was less compared to electric stunning, both at 2 h (3,8% compared to 8,8%) and 7 h (13,8% compared to 18,8%).

From the animal welfare point of view, Velarde et al. (1999) considered that CO_2 stunning was less efficient than electronarcosis with head and side methods, since physiological reflexes, for example corneal reflex and sensibility to pain, with exception to spontaneous respiration, were clearly superior to electric stunning.

The CO_2 stunning caused lactic academia, hyperglycaemia, hyperkalemia, hypercalcemia as well as respiratory and metabolic acidosis in swine, seconds before entering the state of anesthesia. On the other hand, electrocution triggered hypernatremia and hyperglycemia, therefore CO_2 stunning caused major alterations thus possibly compromising animal welfare compared to electrical stunning.

Acknowledgements

This study is from the first author as a member of the Doctorate Program in Biological Science at the Universidad Autónoma Metropolitana Iztapalapa-Xochimilco. Marcelino Becerril-Herrera was supported by the scholarship from CONACYT, Mexico. María Alonso-Spilsbury, Clemente Lemus-Flores, Isabel Guerrero-Legarreta, Ramiro Ramírez-Necoechea and Daniel Mota-Rojas, were supported, as members, by the Sistema Nacional de Investigadores (SNI) in Mexico.

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