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# Aversion to nitrogen and carbon dioxide mixtures for stunning pigs

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## Abstract

*Inhalation of concentrations greater than 30% of carbon dioxide ($CO_2$) by volume in atmospheric air causes aversion in pigs. The objective of this study was to assess, using aversion learning techniques and behavioural studies, the aversion to three alternative gas mixtures of nitrogen ($N_2$) and $CO_2$: 70% $N_2$ and 30% $CO_2$ (70N30C), 80% $N_2$ and 20% $CO_2$ (80N20C) and 85% $N_2$ and 15% $CO_2$ (85N15C). The experiment consisted of two trials of three groups of ten pigs each. Pigs were placed individually at the starting point of the test facility and allowed to enter the crate of a dip-lift stunning system during one control session with atmospheric air and three treatment sessions with one of the gas treatments in each group. When the pit contained any of the three gas mixtures, the time taken to cross the raceway and enter the crate did not increase compared to the control session. However, when exposed to the gas mixtures, the majority (85.80%) of pigs performed attempted retreats in the crate, 22.22% exhibited escape attempts, and 7.91% vocalised, without differences between gas mixtures. The percentage of pigs gasping was higher when exposed to 70N30C compared to 80N20C and 85N15C. The results suggest that pigs show signs of aversion to the inhalation of 15 to 30% $CO_2$ in nitrogen atmosphere compared to atmospheric air but the aversion response did not increase in consecutive sessions.*

**Keywords**: *animal welfare, aversion, carbon dioxide, nitrogen, pigs, stunning*

## Introduction

To avoid pain, suffering or stress, animals must be rendered unconscious prior to being slaughtered (Council Regulation [EC] No 1099/2009). The most commonly used methods for stunning pigs are inhalation of high concentrations of carbon dioxide ($CO_2$) and electronarcosis. When inhaled at high concentrations, $CO_2$ induces hypercapnic hypoxia and depresses brain activity until loss of consciousness (Gregory *et al* 1987; Raj & Gregory 1996; Raj 1999). Carbon dioxide stunning allows exposure of animals in a group, thereby reducing human contact during handling and decreasing pre-slaughter stress (Velarde *et al* 2000). This, together with the lower intensity of muscular contractions compared to electrical stunning, reduces the incidence of pale, soft and exudative (PSE) meat and haemorrhage (Velarde *et al* 2000) and improves meat quality.

Regarding animal welfare, the inhalation of $CO_2$ has been criticised due to its aversive effects prior to loss of consciousness, which is not immediate (Raj *et al* 1997; Rodriguez *et al* 2008). Aversion could be due to inhalation of the gas or the descending movement itself (Hartung *et al* 2002). Previous studies have shown that either isolation of an animal and caging (Raj & Gregory 1996), or the vertical movement of a crate descending into atmospheric air (EFSA 2004) induces fear in pigs. However, this aversive reaction to the descent of the stunning box decreases when pigs are exposed repeatedly to this situation (Velarde *et al* 2007; Dalmau *et al* 2010b). In humans, inhalation of high concentrations of $CO_2$ causes irritation of the respiratory tract and a sense of breathlessness (Gregory *et al* 1990). Raj and Gregory (1995) assessed aversion from the pigs' reluctance to enter into different gaseous atmospheres to obtain a reward (apples), and concluded that an atmosphere containing 90% $CO_2$ was aversive to the majority of pigs. $CO_2$-sensitive chemoceptors are distributed along the respiratory tract (Manning & Schwartzstein, 1995) and their activation in the upper tract causes anxiety and pain in pigs (Troeger & Waltersdorf 1991). Velarde *et al* (2007) suggested that the higher the $CO_2$ concentration, the more pronounced the aversion. Additionally, $CO_2$ induces severe respiratory distress causing hyperventilation associated with gasping (Gregory *et al* 1990). Gasping is a rudimentary respiratory activity occurring through the mouth, indicative of a sense of breathlessness (EFSA 2004). Some results indicate that when pigs perceive the gas, signs of aversion, such as backing away, head-shaking and attempting to escape are observed (Raj & Gregory 1996).



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In contrast to hypercapnia, hypoxia (less than 2% $O_2$ by volume in atmospheric air) induced by the inhalation of an atmosphere with inert gases, such as argon (Ar) or nitrogen ($N_2$), that displaces oxygen, is reported to be non-aversive (Ernsting 1963, 1965; Raj *et al* 1997; Raj 1999). Research has shown that argon-induced hypoxia does not induce aversion or any signs of respiratory distress prior to loss of consciousness (Raj & Gregory 1995). However, argon has a low presence in the atmosphere (0.9%) and its availability for commercial stunning practices might be limited (Dalmau *et al* 2010a). In contrast, nitrogen is the main component of atmospheric air and its availability is higher than that of other inert gases. However, the time to loss of consciousness when exposed to hypoxia (54 s) is longer than when animals are exposed to hypercapnia (> 80% $CO_2$) (32 s) (Raj *et al* 1997). Gregory (1995) reported that the addition of $CO_2$ to a hypoxic atmosphere reduces the time needed to induce unconsciousness. However, according to Raj and Gregory (1995), the $CO_2$ concentration of the gas mixture should be up to 30% in the atmosphere in order to avoid aversion.

The relative density of nitrogen (0.97) is slightly lower than that of air (1.00) and its stability, defined as the capability of the gas to be sustained within the pit without being displaced by oxygen, is uncertain (Dalmau *et al* 2010a). Nevertheless, this stability could be improved by combining nitrogen with $CO_2$. Hence, the higher the concentration of nitrogen in a gas mixture with $CO_2$, the lower the relative density of the mixture and, therefore, the more difficult it is to displace the oxygen from the pit. In addition, gas mixtures of $N_2$ and up to 30% $CO_2$ have high stability and uniform concentrations along the pit (Dalmau *et al* 2010a).

Aversion learning techniques are based on behaviour assessment, and have been used to objectively determine the degree of aversion that animals experience during short-lasting events. Rushen (1996) proposed that these techniques may be useful in predicting the extent of some of the physiological responses to stressors, which suggests that the aversion that an animal feels in response to a treatment is a factor in determining the magnitude of the physiological response. Raj and Gregory (1995) stated that when pigs are exposed to an unpleasant situation, such as exposure to high concentrations of $CO_2$, avoidance behaviour could be taken as a sign of aversion.

The objective of this study was to assess the aversion of slaughter pigs to the inhalation of 70% $N_2$ and 30% $CO_2$ (70N30C), 80% $N_2$ and 20% $CO_2$ (80N20C) and 85% $N_2$ and 15% $CO_2$ (85N15C) with less than 2% of $O_2$ by volume in atmospheric air.

## Materials and methods

### Study animals

Sixty halothane-free female pigs (93.1 [± 1.91] kg live weight) were used in the experiment. Pigs came from a commercial farm in two separate batches of thirty animals and arrived at the IRTA facilities three days prior to the start of the trial. Upon arrival, each batch was divided into three groups of ten animals, and each group was housed in an 18.3 m$^2$ pen (8.3 × 2.2 m; length × width). Pigs were fed and given water *ad libitum* with the same diet that they had received on the farm of origin.

### Facilities

The experiment was carried out in the experimental slaughterhouse of IRTA, which is equipped with a Dip Lift stunning unit (Butina, Alps, Copenhagen, Denmark). This system consists of a crate measuring 195 × 61 × 90 cm (length × width × height) and with a perforated floor to facilitate gas distribution. The crate has a guillotine entrance door and an exit ramp gate at the opposite end, both with a non-slip steel ramp angled at 7º to facilitate the entry and exit of animals. The crate descends into a well of 260 cm depth and 8 m$^3$ in area. The required gas mixtures were supplied through an inlet valve placed at the bottom of the pit. The $N_2$ and $CO_2$ concentrations were mixed and controlled with two flowmeters (Dalmau *et al* 2010a,b) that worked at three bars of pressure and a flow rate of 16 Nm$^3$ h$^{-1}$. Prior to each exposure, the $CO_2$ and $O_2$ concentrations were monitored at 120-cm depth with a portable infrared and electrochemical sensor (Map Check Combi $O_2$/$CO_2$, PBI-Dansensor, Spain), and pre-filled until desired concentrations were reached. The building containing the housing pens was adjacent to the slaughterhouse and connected to the stunning unit by a corridor (412 × 60 cm; length × width). Corridor walls consisted of stainless steel panels at a height of 90 cm which prevented pigs from seeing over the top and turning around.

### Experimental procedure

The experiment consisted of two similar trials with three groups of ten animals each. Each trial included four sessions: one control session and three treatment sessions. The sessions were carried out daily with an average interval of 24 h in order to give recovery time between gas exposures. Adequate recovery was assessed prior to exposure by means of alimentary, exploratory and resting behaviour in the pen and any individual showing failure to recover was removed from the experiment. During the build-up to the treatment sessions, animals were trained for four consecutive days to familiarise them with the stunning facilities and reduce the novelty effect until two days prior to the control session. In the first two training sessions, pigs were moved through the corridor and the stunning crate, which remained stationary, to allow the animal to move through freely. After leaving the crate, pigs were taken back to the housing pen. During the third and fourth training sessions, after entering the crate, the animals were lowered into the pit with atmospheric air. The control session was taken as a reference for the pigs' behaviour in atmospheric air and compared with subsequent gas-mixture treatments. During treatment sessions, the first group was exposed to a gas mixture containing 70N30C; the second group exposed to a gas mixture containing 80N20C, and the third to a mixture of 85N15C. In each group, the well contained one of the three gas mixtures during all treatment sessions.

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During control and treatment sessions, pigs were taken individually, in a random order within the group, to the start of the corridor and allowed to cross it for 10 min until they entered the crate. If, after that time, an animal had not entered the crate, a person placed behind prevented it from coming back. Five minutes later, if the pig was still reluctant to enter into the crate, it was pushed gently inside and the guillotine door closed. When the animal entered the crate, the exit gate was closed and the pig lowered into the well. During training, control and treatment sessions, pigs were lowered into the well with atmospheric air for 15 s until a depth of 120 cm was attained, they then remained stationary for 5 s before ascending for 15 s. The total cycle time was 35 s. Once at the top, the exit gate was opened and the pig returned to the housing pen. Once the cycle was finished, the animal was allowed to recover and, afterwards, moved to the housing pen. Prior to the following pig's exposure, faeces and urine — if present — were removed from the corridor and the inside of the crate. After the end of each group-treatment sessions all pigs were euthanised by exposure to 90% concentrations of $CO_2$.

### Measurements

The behaviour of each animal during training and treatment sessions was recorded by two video cameras, one placed in the corridor area and the other inside the crate. Both video cameras were connected to a digital audio-image recorder (VDVR-4S 550430, Circontrol, Spain).

In the corridor, the time each pig took to enter the crate was recorded. In addition, handling was scored as '0' if the pig entered the crate voluntarily or '1' if not and it had to be pushed into the crate. The presence and onset of the following behaviours in the crate were assessed to determine aversion:

• Retreat attempts — pigs backing away (Dodman 1977; Dalmau *et al* 2010b);

• Escape attempts — pigs raising their forelegs on the side of the crate either at the time of or prior to loss of balance (Raj & Gregory 1996);

• Vocalisation — shouts or snores emitted by the animal during induction of unconsciousness (EFSA 2004; Rodríguez *et al* 2008), only those emitted prior to loss of balance were considered a sign of aversion; and

• Gasping — a very deep breath through a wide open mouth, which may involve stretching of the neck: considered to be an indicator of onset of breathlessness (Velarde *et al* 2007).

The presence and duration of muscular excitation, defined as a period of struggling ranging from fairly vigorous running and jumping movements to clonic convulsive seizures (Dodman 1977), were recorded.

The time to loss of balance, defined by the inability of the animal to remain in a standing position, was considered the first indicator of the onset of unconsciousness (Raj & Gregory 1996). All behaviours in the corridor and crate were assessed visually by means of the video recording whereas vocalisations were assessed via audio recordings. All recording times were synchronised with the time the crate started to descend into the pit.

### Statistical analysis

Data were analysed with the Statistical Analysis System package (SAS 9.1, SAS Institute Inc, Cary, NC, USA 1999-2001). Time to cross the corridor, to show retreat attempts, to show escape attempts, to show gasping, to loss of balance, to show vocalisations and the onset of the muscular excitation phase as well as its duration, were analysed using a mixed model analysis of variance (PROC MIXED) with a covariance structure of compounds symmetric (CS) using 'trial', 'gas mixture' and 'session' as fixed effects. When differences between trials were significant ($P < 0.05$), each trial was analysed separately. The order of the animal as a random effect and the animals as repeated measures were included in the model. When the analysis of variance showed significant differences ($P < 0.05$), a least square means comparison test (LSMEANS) adjusted to multiple comparison test of Tukey was carried out.

Binary data, such as presence of retreat attempts, escape attempts, loss of balance, gasping, vocalisations and muscular excitation were analysed using a generalised linear model analysis of variance (PROC GENMOD) following a binomial distribution. 'Trial', 'session' and 'gas mixture' were considered as fixed effects.

During the control session, differences among groups were analysed. The behaviour during the treatment sessions was compared with that during the control session. Different correlations (PROC CORR) were analysed between those variables that could have an effect according to the aversion criteria using Pearson's correlation coefficient (parametric data) or Spearman rank coefficient (non-parametric data).

This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of IRTA.

## Results

### Control session

Time to enter the crate (249.3 [± 35.19] s) and the percentage of animals that entered voluntarily (84.8 [± 4.72]%) did not differ between trials or groups. During the descent of the crate into the pit, the percentage of animals attempting to retreat (67.8 [± 6.13]%) and attempting to escape (5.0 [± 2.83]%) did not differ between trials or groups. In the control session, none of the pigs showed gasping, loss of balance, vocalisations or muscular excitation inside the crate.

### Treatment sessions

The time to enter the crate and the percentage of animals not entering voluntarily were significantly higher ($P = 0.0037$) in Trial 1 (388.2 [± 30.57] s and 30%, respectively) than in Trial 2 (223.5 [± 15] s and 11%, respectively). Nevertheless, none of these measures increased significantly between control and treatment sessions in any gas mixture.

The percentage of animals that showed signs of aversion in the crate that were significantly different between sessions and treatments is presented in Table 1. The chronological onset of these behaviours in the crate is shown in Figure 1.



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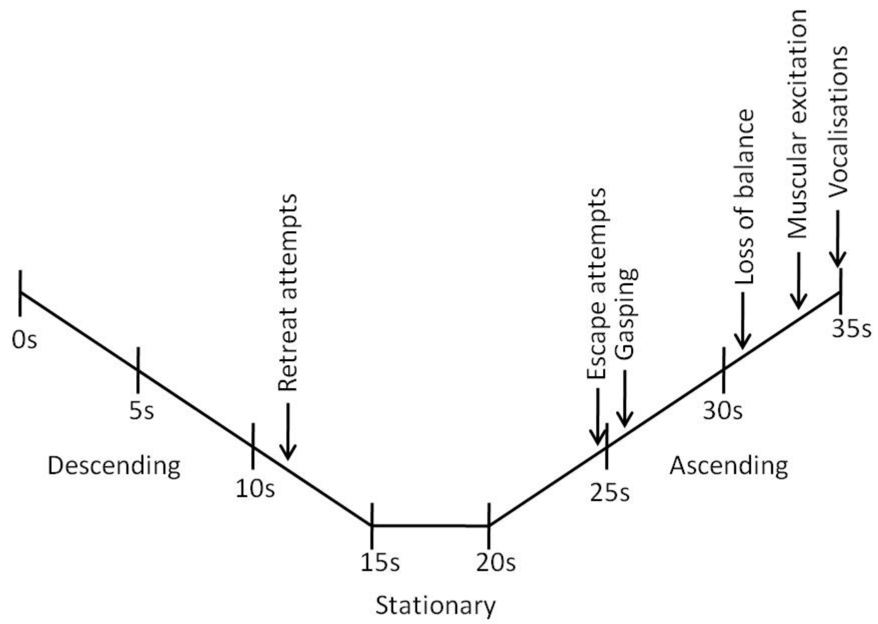
**Table 1 Percentages of animals showing retreat attempts, escape attempts, vocalisations, muscular excitation and gasping by treatments 70N30C, 80N20C and 85N15C.**

| Factor | Session | 70N30C | 80N20C | 85N15C |
|---|---|---|---|---|
| Retreat attempts (%) | Control | 73.68 | 60.00 | 70.00 |
| | 4 | 73.68 | 85.00 | 85.00 |
| | 5 | 100 | 90.00 | 85.00 |
| | 6 | 84.21 | 95.00 | 73.68 |
| Escape attempts (%) | Control | $0^{b}$ | $10.00^{b}$ | $5.00^{b}$ |
| | 4 | $20.00^{a}$ | $15.00^{a}$ | $15.00^{a}$ |
| | 5 | $30.00^{a}$ | $40.00^{a}$ | $20.00^{a}$ |
| | 6 | $25.00^{a}$ | $25.00^{a}$ | $10.00^{a}$ |
| Vocalisations (%) | Control | – | – | – |
| | 4 | 31.57 | 35.00 | 40.00 |
| | 5 | 42.10 | 50.00 | 40.00 |
| | 6 | 42.10 | 35.00 | 30.00 |
| Muscular excitation (%) | Control | – | – | – |
| | 4 | $26.31^{x}$ | $5.00^{y}$ | $20.00^{x}$ |
| | 5 | $35.29^{x}$ | $5.00^{y}$ | $30.00^{x}$ |
| | 6 | $36.84^{x}$ | $15^{y}$ | $25^{x}$ |
| Gasping (%) | Control | – | – | – |
| | 4 | $57.89^{x}$ | $35.00^{y}$ | $15.00^{y}$ |
| | 5 | $78.95^{x}$ | $30.00^{y}$ | $50.00^{y}$ |
| | 6 | $78.94^{x}$ | $55.00^{y}$ | $40.00^{y}$ |
| Loss of balance (%) | Control | – | – | – |
| | 4 | 52.63 | 65 | 55 |
| | 5 | 68.42 | 65 | 55 |
| | 6 | 73.68 | 80 | 65 |

$^{a, b, c}$ Means with different superscripts differ significantly at $P < 0.05$ between sessions.
$^{x, y, z}$ Means with different superscripts differ between treatments at $P < 0.05$.

**Figure 1**




Average time to perform retreat and escape attempts, gasping, loss of balance, muscular excitation and vocalisations during gas exposure.



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In all gas mixtures, the percentage of animals that performed attempted retreats during treatment sessions increased compared to the control session, being significantly higher in the last treatment session ($P = 0.0141$) (Table 1). The time to show the first retreat attempt was not affected by gas mixture or session in any trial (10.8 [± 0.60] s). Animals that did not enter the crate voluntarily exhibited fewer retreat attempts than those that entered voluntarily ($P = 0.0346$). In the three gas mixtures, the percentage of animals that performed escape attempts during the treatment sessions was higher compared to the control session ($P < 0.05$). The time to perform the first escape attempt did not differ between gas mixtures or session in any trial (24.2 [± 1.40] s). Vocalisations, muscular excitation, gasping and loss of balance appeared only during treatment sessions. The prevalence of gasping was significantly ($P < 0.001$) higher in 70N30C compared to 80N20C and 85N15C (71.93 vs 40.00 and 35.00%, respectively) and no differences were found in the time to perform these between gas mixtures and sessions (25.7 [± 0.72] s). On average, 7.91% of pigs vocalised prior to loss of balance at 30.2 (± 2.71) s after the beginning of the exposure, with no differences between gas mixtures and sessions. The percentage of animals that performed muscular excitation was significantly lower when exposed to 80N20C (8.33%) than when exposed to 70N30C (32.73%, $P = 0.002$) and 85N15C (25.00%, $P = 0.0186$). The time to onset of muscular excitation (34.9 [± 0.72] s) and its duration (8.9 [± 0.82] s) were not affected by the gas mixtures or sessions in any trial.

The percentage of animals that lost their balance (64.41%) was not affected by gas mixtures or sessions, but was significantly higher in pigs that had been pushed into the crate compared to those entering voluntarily (100 vs 63.24%, respectively; $P < 0.001$). In the second trial, pigs exposed to 85N15C lost balance earlier than those exposed to 80N20C (29.2 [± 0.61] vs 32.2 [± 0.43], respectively; $P = 0.0089$).

## Discussion

Velarde *et al* (2007) and Dalmau *et al* (2010b), using the same facilities used in this study, determined that pigs became habituated after being repeatedly exposed to the process of entering the crate and descending into a pit with atmospheric air. When the control session started, pigs were already habituated to the test facilities and the movement of the crate through the pit. Hence, it was decided to follow the same experimental design as Velarde *et al* (2007) and establish the control session after two training sessions, in order to compare air and treatment gas mixtures.

Velarde *et al* (2007) stated that increased time to enter the crate when pigs are exposed to an unpleasant situation on consecutive sessions indicates aversion. Actually, animals can learn to predict from certain stimuli, such as crossing the corridor that leads to the gas exposure, and if it is negative, the animals show aversion to the stimulus in question. The magnitude of these responses can be measured objectively by measuring the time to enter and this can be used to infer the extent of aversion (Rushen 1986). According to our results, the time to enter the crate and the percentage of animals not entering the crate voluntarily did not increase significantly between control and treatment sessions. Hence, in accordance to the magnitude of the aversive response, the exposure to nitrogen and $CO_2$ mixtures was not unpleasant enough for animals trying to avoid it in subsequent sessions. Dalmau *et al* (2010b) found that pigs that had shown muscular excitation during gas exposure in previous sessions increased significantly the aversive response in subsequent exposures. They concluded that the muscular excitation suffered by some animals during the gas exposure causes trauma and pain which, by association, leads to an increased time to enter the crate in subsequent sessions. According to our results, pigs exposed to 70N30C and 85N15C showed a higher incidence of muscular excitation than pigs exposed to 80N20C. However, the reluctance to enter the crate in subsequent sessions did not differ between treatments. In contrast to Dalmau *et al* (2010b), we never found signs of trauma or pain during treatment sessions, and they used longer exposure times to gas treatment and for more sessions than in the present study. It has also been stated that an increase in $CO_2$ concentration stimulates aversion to the gas mixture due to early detection of the gas at the entrance of the crate (Velarde *et al* 2007). Our results suggest that at between 15 to 30% $CO_2$, pigs are unable to distinguish differences in $CO_2$ concentrations before entering the crate.

Dodman (1977) and Raj and Gregory (1996) considered both retreat and escape attempts as signs of aversion. Although those behaviours were also present during the control session, the percentage of animals showing retreat and escape attempts was higher in the treatment sessions compared to the control, suggesting that the exposure to any of the gas mixtures of the study could be more aversive for pigs than the exposure to atmospheric air. In fact, the appearance of gasping and vocalisations in some animals before the loss of balance during the treatment sessions confirms that exposure to nitrogen mixed with 15 to 30% $CO_2$ is aversive compared to atmospheric air, as suggested by Dalmau *et al* (2010b). Regarding the high concentrations of $CO_2$, Dalmau *et al* (2010b) compared the time to retreat between pigs exposed to hypercapnic anoxia (70N30C and 85N15C) with the results obtained by Velarde *et al* (2007) in pigs exposed to 90% $CO_2$ (90C) and 70% $CO_2$ (70C) in the same facilities and concluded that the first retreat attempt appeared earlier in animals exposed to 90C and 70C than in animals exposed to 70N30C or 85N15C. The time to retreat in our experiment confirms these findings since it did not differ between gases (11.4 [± 0.69] s), and the first retreat attempt appeared later than in animals exposed to 70C and 90C. In conclusion, our results show that aversion to $CO_2$ gas mixtures is higher than to atmospheric air but lower than to high concentrations of $CO_2$.

Raj (1999) suggested that vocalisations after loss of balance could occur while the animals were unconscious and consequently could not be taken as signs of aversion. In this study, 8% of pigs exposed to nitrogen and $CO_2$ mixtures



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vocalised prior to the loss of balance. The fact that this measure was not present during the control session and appeared during treatment sessions suggests that at least 8% of the pigs felt the inhalation of the gas mixtures aversive. A more accurate determination of the state of awareness before and after the loss of balance is needed to confirm if vocalisations after the loss of balance are signs of aversion.

According to the prevalence and onset of retreat attempts, escape attempts and vocalisations, the aversive response to the inhalation was similar regardless of the gas mixture. These results are in agreement with Dalmau *et al* (2010b), who compared different nitrogen and $CO_2$ mixtures with high concentrations of argon, and concluded that aversion only decreased significantly using high concentrations of argon without $CO_2$ in the atmosphere. The incidence of gasping was higher in animals exposed to 70N30C than in animals exposed to 80N20C and 85N15C. Although gasping is not an expression of aversion, it is a rudimentary respiratory activity occurring through the mouth, and considered a physiological reaction associated with breathlessness during the inhalation of $CO_2$ (Raj & Gregory 1996). Raj and Gregory (1996) stated that inhalation of more than 30% of $CO_2$ induces severe respiratory distress. According to our results, this also applies to mixtures with around 30% $CO_2$ but become significantly lower at concentrations around 20%.

Loss of balance is considered the first indicator of onset of unconsciousness (Raj & Gregory 1996). The proportion of animals that lost balance was similar between gas mixtures. Nevertheless, the time to loss of balance was shorter in the gas mixture with the lowest $CO_2$ concentration (85N15C) than in 80N20C during the second trial. In contrast, Dalmau *et al* (2010b) and Raj *et al* (1997) suggested that the higher the $CO_2$ concentration, the shorter the time to lose balance, and consequently, consciousness. In our experiment, this difference appeared only in the second trial, making this result unreliable. In fact, during this trial, only 53% of the animals exposed to 85N15C lost their balance compared to 83% of the animals exposed to 80N20C and it is therefore difficult to draw conclusions. On the other hand, an effect of handling to enter the crate was found since pigs that were pushed into the crate lost their balance earlier than those entering voluntarily. Indeed, Broom (2000) reported that a higher excitability in pigs could produce an increase in the respiratory frequency with faster and deeper respirations, which facilitates the uptake of $CO_2$ and shortens the induction of unconsciousness (Forslid 1992). Hence, as mentioned previously by Dalmau *et al* (2010b) and Velarde *et al* (2007), a higher excitation of the animals that entered the crate reluctantly may modify the effect of the gas mixtures on the animal.

## Animal welfare implications and conclusion

Taking into account the time to enter the crate and the percentage of animals entering voluntarily, it is concluded that exposure to nitrogen and carbon dioxide mixtures does not represent a negative stimulus that animals try to avoid in consecutive sessions. Based on the percentage of animals showing retreat and escape attempts, gasping and vocalisations before the loss of balance, it can be concluded that the exposure to 70N30C, 80N20C and 85N15C is more aversive than exposure to atmospheric air. Pigs show a similar aversion to the three gas mixtures although a higher $CO_2$ concentration in the atmosphere causes an increase of the sense of breathlessness. Pigs exposed to 80N20C show less muscular excitation during exposure to the gas.

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