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Stunning pigs with different gas mixtures: aversion in pigs

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Abstract

The objective of this study was to assess the aversion to exposure of 90% argon, 70% N_2/30% CO_2 and 85% N_2/15% CO_2 by volume in atmospheric air in 24 halothane-free slaughter-weight pigs using aversion learning techniques and behavioural studies in an experimental slaughterhouse. Pigs were subjected to the treatments individually during 2 separate trials of 12 animals each. The time of exposure to the gases was 46 and 32 s, respectively. When the pit contained any of the 3 gas mixtures, the time taken to cross the raceway and enter the cradle (TCREC) increased compared with the training sessions (atmospheric air). The incidence of pigs showing retreat and escape attempts and gasps and the number of times that this behaviour was performed was lower in 90% argon than in the gas mixtures with N_2 and CO_2. On the other hand, the time to loss of posture was lower with 70% N_2/30% CO_2 than with argon. The second exposure to all gas mixtures was more aversive than the first and the loss of posture also occurred earlier in the second exposure. In conclusion, pigs showed more aversion to gas mixtures with N_2 and either 15% or 30% CO_2 by volume than 90% argon by volume.

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

Introduction

Stunning before slaughter is a statutory requirement in Europe (EC 2009) and is performed to induce unconsciousness and insensibility in animals so that the slaughter can be performed without causing the animals any avoidable anxiety, pain, suffering or distress. Under commercial conditions, two main methods are used to stun pigs (*Sus scrofa*): electrical and carbon dioxide (CO_2) stunning.

The use of CO_2-stunning systems has increased in popularity due to their positive effects on meat quality compared with electrical stunning (Velarde *et al* 2000, 2001). In the CO_2-stunning system, pigs are loaded into a cradle which is then lowered into a pit pre-filled with a high concentration of CO_2. However, its acceptability on welfare grounds has been questioned. Firstly, animals may be stressed when they are loaded into the cradle and lowered into the pit whether it contains CO_2 or atmospheric air. Secondly, loss of consciousness is not immediate and pigs react aversely to exposure with a concentration above 30% CO_2 (Raj & Gregory 1995; Velarde *et al* 2007). Inhalation of CO_2 at high concentrations causes irritation in the nasal mucosal membranes and lungs (Peppel & Anton 1993), where the presence of chemoceptors acutely sensitive to this gas has been described (Manning & Schwartzstein 1995). Additionally, CO_2 induces severe respiratory distress causing hyperventilation and a sense of breathlessness during the induction phase prior to loss of consciousness (Gregory *et al* 1990). In fact, aversive behaviour during the induction of unconsciousness with CO_2 has been described in pigs. When pigs are presented with an unpleasant situation their first reaction is to retreat from the situation which is associated with the noxious stimulus (Dodman 1977). Raj and Gregory (1995) reported that pigs tried to escape from an atmosphere of 90% CO_2 in less than 5 s. Troeger and Woltersdorf (1991) observed that immediately after immersing pigs into the CO_2 gas mixture (60 to 90%), they backed away and sniffed. Before loss of posture, pigs show vigorous head shaking (EFSA 2004), a large gasp through the wide open mouth, which is indicative of the onset of breathlessness, and escape attempts (Raj & Gregory 1996).

Hypoxia, induced by the inhalation of inert gases, such as argon or nitrogen, has also been evaluated to stun pigs under experimental conditions (Raj & Gregory 1995, 1996; Raj 1999). In contrast to hypercapnia, research has shown that hypoxia or anoxia does not cause aversion in pigs and does not induce any signs of respiratory distress prior to loss of consciousness (Raj & Gregory 1995).

Aversion learning tests help to uncover what animals find unpleasant (fear, discomfort and pain). These techniques have been used by several authors to test the aversiveness of handling methods and other treatments and rely on the fact that animals remember aversive situations in repeated exposures to such situations. In studies by Rushen (1986) and Rushen and Congdon (1987) with sheep, and Jongman *et al* (2000) and Velarde *et al* (2007) with pigs, animals

Table 1 Experimental protocol carried out during Trials 1 and 2 for the exposure of pigs to 90% argon by volume in atmospheric air (AR) and the mixtures 70% N_2 and 30% CO_2 by volume in atmospheric air (70N30C) and 85% N_2 and 15% CO_2 (85N15C).

Session number	Trial 1	Trial 2
	Time of exposure, 46 s	Time of exposure, 32 s
Training session 1	Atmospheric air	Atmospheric air
Training session 2	Atmospheric air	Atmospheric air
Training session 3	Atmospheric air	Atmospheric air
1	70N30C	AR
2	85N15C	70N30C
3	AR	85N15C
4	AR	85N15C
5	85N15C	70N30C
6	70N30C	AR
7	85N15C	70N30C
8	AR	85N15C
9	70N30C	AR

were repeatedly exposed to different treatments and the time to re-enter the treatment area was assessed.

The objective of this study was to ascertain whether 90% argon (AR) by volume in atmospheric air and gas mixtures of 70% N_2 and 30% CO_2 (70N30C) and 85% N_2 and 15% CO_2 (85N15C) by volume in atmospheric air in a commercial dip-lift stunning system are aversive to slaughter-weight pigs by means of the study of aversion-learning tests and the behaviour of pigs in the pit when exposed to the gas mixtures.

Materials and methods

Animals

Twenty-four halothane-free female pigs were transported from a commercial farm to the experimental slaughterhouse at IRTA-Monells, Girona, Spain in two batches of 12 animals in two separate trials carried out during the experiment. On arrival, each batch was divided into two groups of six pigs and housed in adjacent lairage pens (4 × 2 m; length × breadth) next to the experimental slaughterhouse. The pigs were fed *ad libitum* with the same diet as on the original farm, and water was also made available *ad libitum*. The mean live weight of the pigs on the last day of the experiment was 104.6 (± 2.30) and 99.0 (± 2.20) kg for first and second batch, respectively.

Experimental procedure

The test facility consisted of a raceway and a dip-lift stunning unit. The raceway from the housing pens to the stunning unit was 412 × 60 cm (length × breadth), allowing movement but preventing the animal from turning around. It was bordered by 90-cm high steel panels to prevent the animal seeing over the top. The raceway led to the CO_2-stunning unit cradle by a non-slip steel ramp of 148-cm length and with a slope of 7º. The dip-lift stunning system (Butina Alps, Copenhagen, Denmark) contained a cradle (195 × 90 × 61 cm; length × breadth × height). The cradle was provided with an entrance guillotine gate at the end of the raceway and an exit ramp gate at the other end. The floor of the cradle was perforated to facilitate the distribution of the gas inside, so pigs could sniff small concentrations of the gas mixtures while they were at the end of the raceway and in the cradle. On closing the gate, the cradle descended to the base of a 260-cm deep well with a capacity of 8 m^3. The required gas mixture concentrations were supplied through an inlet valve placed at the bottom of the pit. The CO_2, N_2 and argon concentrations of each gas mixture were controlled and mixed with two flowmeters (Dalmau *et al* 2010) that worked at three bars of pressure and a flow rate of 16 Nm^3 $hour^{-1}$. The gas mixture concentrations were monitored from the concentration of CO_2 and O_2 with a portable infrared and electrochemical sensor, respectively (Checkpoint O_2/CO_2, PBI Dansensor A/S, Denmark).

Both separate trials consisted of three training and nine treatment sessions (Table 1). During the training sessions the stunning unit contained atmospheric air whereas during the treatment session the stunning unit was filled with AR, 85N15N or 70N30C, according to Table 1. Each day included two sessions, from 0900 to 1300h in the morning and 1500 to1900h in the afternoon.

One hour before the start of each session, both groups of six animals were moved to two separate waiting pens where only water was available. The pigs were marked for individual identification and the group order to start was changed every two sessions. In random order, pigs were placed individually at the starting point of the raceway and allowed to cross the raceway and enter the stunning cradle for 10 min. If, after that time, the pig was reluctant to enter the cradle, a person was placed behind the animal to avoid backward movements after advancing. If, 5 min later, the pig was still reluctant to enter the cradle, it was gently pushed into the cradle.

After the pig entered the cradle, the gate was closed and it descended to the bottom of the pit for 23 s in Trial 1 and 16 s in Trial 2 and then ascended for 23 s in Trial 1 and 16 s in Trial 2. The total cycle lasted 46 and 32 s in the first and second trials, respectively. When the cradle reached the top position, the exit gate was opened and the pig was allowed to go out of the stunning unit and return to the housing pen, where food was provided. In each group, the animals that took the most time to enter the cradle during the last two training sessions were discarded for the treatment sessions and used for replacement. At the end of each treatment session, the replacement pigs entered and exited the cradle but without descending to the bottom of the pit. In the first trial, one pig was replaced after the seventh session. No animals were replaced during the

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second trial. Therefore, 11 pigs were used for the treatment sessions in Trial 1 and 10 in Trial 2.

Pigs were individually exposed during three different sessions to each gas mixture according to the protocol of Table 1.

Measurements

Aversion-learning measurements were taken to assess the aversion to the stunning system with either atmospheric air or the different gas mixtures. The time taken to cross the raceway and enter the cradle (TCREC) was recorded for each animal with a video camera placed at the starting point of the raceway. The TCREC ended when the tail of the pig crossed the gate and the gate was closed. The handling of the animals was scored as 0 if the pig entered the cradle voluntarily or 1 if it did not. The behaviour of the pigs during the descent and ascent in the pit was also recorded with a second video camera placed on the roof of the cradle. The behavioural parameters scored in the cradle to determine the aversion were:

• Retreat attempts: pigs backing away (Dodman 1977);

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

• Escape attempts: pigs running across the cradle and/or raising their forelegs on the side wall of the cradle (Raj & Gregory 1996).

Muscle jerks, defined as muscular contractions similar to spasms or convulsions (Forslid 1987; Raj & Gregory 1995) in the whole body or part of it, were considered and their onset, duration and intensity recorded. The intensity was scored by the observer as: 0) absence; 1) low; and 2) high. The time to loss of posture, considered the first indicator of onset of unconsciousness (Raj & Gregory 1996), was also recorded. Finally, the onset and number of gagging movements (very deep breath together with stretching of the neck when the animal was lying down; Raj 1999) were scored. All recording times were synchronised with the time the pigs started to descend into the well.

Observations were time intervals (TCREC, time to first retreat and to attempt escape, time to first gasp, time to loss of posture, time of onset of muscle jerks and their duration, and time of first gagging movement), binary data (handling and presence of loss of posture) and count data. For count data, as more than 80% of the pigs performed less than 2 retreat attempts, gasps, escape attempts or gagging movements, these variables were scored in three categories: 0 events (0); one event (1); and two or more events (2).

Other variables considered in the study were group of the animal, session number and number of exposures to the gas mixture (first, second or third).

Statistical analysis

Analyses were carried out with the Statistical Analysis System (SAS software, SAS Institute Inc, Cary, NC, USA; 1999-2001). General linear models (PROC MIXED) with animal as a repeated measure were used to analyse the dependent variables TCREC, time to perform the first retreat attempt, gasp, escape attempt and gagging movement, time taken to lose posture and time to start and duration of muscle jerks. The fixed effects included in the model were the gas mixture, the trial and their interaction. For TCREC, the model also took into account as independent variables the number of gasps, retreat attempts, escape attempts and the intensity of muscle jerks during the previous session. In addition, the session number and the group of animals were also considered as independent variables in the TCREC model. When the analysis of variance showed significant differences ($P < 0.05$), the least square means of fixed effects (LSMEANS) adjusted to Tukey's honestly significant difference (HSD) was used to carry out the multiple comparison.

Intensity of muscle jerks, number of retreat attempts, gasps, escape attempts and gagging movements were analysed by the PROC GENMOD procedure of SAS with animal as repeated measure. The fixed effects included in the model were gas mixture, session and trial. A multinomial distribution with a cumulative logit model as link function was used in all cases. The estimate statements were used to analyse the gas mixtures, sessions and trials in each fixed effect. Two types of statistical analyses were used, one including both trials in the model, and another analysing each trial separately. When a trial effect was observed in the general model with both trials, each trial was considered separately. In these cases, results from LSMEANS are only shown for analysis of each trial separately. In all cases, significance was fixed at $P < 0.05$.

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

Results

Training sessions

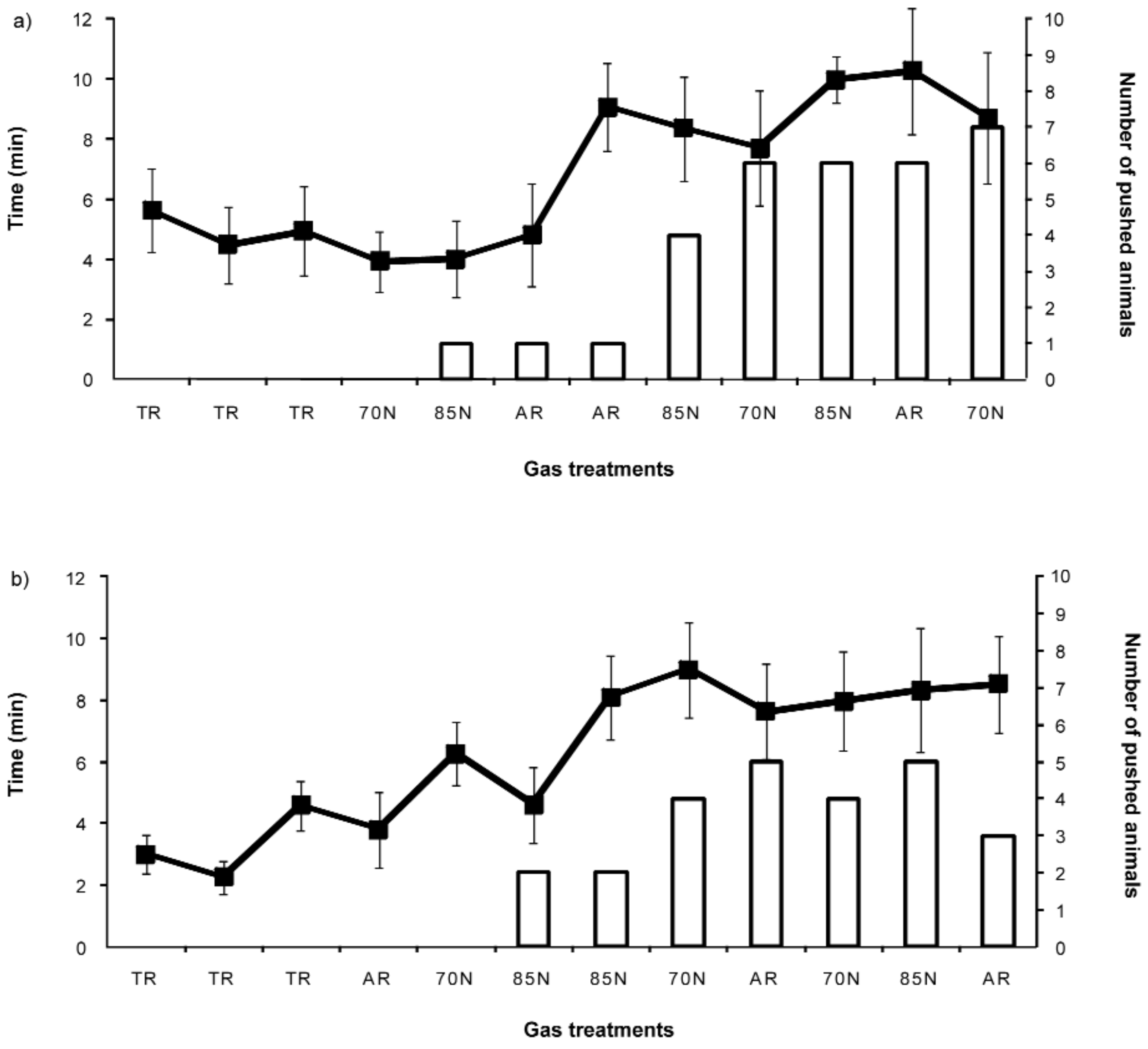
No differences were found in TCREC during the training sessions between both trials or between the different training sessions within each trial ($P > 0.05$; Figure 1).

Treatment sessions

TCREC and the number of animals that needed to be pushed into the cradle were higher ($P < 0.001$) during the treatment sessions than during training sessions in both trials. The time for the first retreat attempt to appear in Trial 2 was lower ($P < 0.001$) during the training (4.8 [± 1.48]) than treatment (11.5 [± 1.11]) sessions.

In the treatment sessions, the number of escape attempts, the time to perform the first gasp and number of gasps did not differ ($P > 0.05$) between trials. In both trials, the proportion of pigs that showed escape attempts was lower ($P < 0.05$) in AR than in 70N30C and 85N15C (Table 2). The time to perform the first gasp was higher ($P = 0.0071$) during the first exposure than the second. The percentage of pigs that did not gasp was higher ($P < 0.0001$) in AR than in 70N30C and 85N15C (Table 2) and during the first exposure than during the second exposure to each gas mixture ($P < 0.05$).

Figure 1



Mean (± SEM) time taken to cross the raceway and enter the cradle in the two trials (in lines), considering only those animals that entered the system of their own accord, without being pushed, and the number of animals being gently pushed (in bars) in each session. Training sessions (TR), 85% N_2 and 15% CO_2 by volume in atmospheric air (85N), 70% N_2 and 30% CO_2 by volume in atmospheric air (70N), and 90% argon by volume in atmospheric air (AR). Different letters represent significant differences at $P < 0.05$.

Trial 1

TCREC was higher ($P = 0.0159$) in 70N30C than in AR.

The number of retreat attempts was higher ($P < 0.01$) in 85N15C compared with the other gas mixtures (Table 2). The time to loss of posture was lower ($P = 0.0172$) in 70N30C than AR (Table 3), and the proportion of animals that lost their posture before the onset of muscle jerks was lower ($P < 0.05$) in AR than in 70N30C and 85N15C (Table 4). The intensity of muscle jerks was lower ($P < 0.0001$) in AR than in 70N30C and 85N15C (Table 5). The amount of gagging was higher ($P < 0.05$) in 70N30C than in AR and 85N15C and higher in 85N15C than in AR ($P = 0.0009$; Table 5).

Muscular jerks appeared earlier ($P < 0.0001$) during the third exposure to the gas treatments than during the second. The time taken to perform the first retreat attempt was affected by the number of escape attempts, in which animals with two or more escape attempts showed this behaviour earlier ($P = 0.0491$) than animals with only one escape attempt. The time to loss of posture was affected by gasping, in which animals with this behaviour lost posture earlier ($P = 0.0066$) than animals without gasps. The intensity of muscle jerks was higher ($P < 0.01$) in animals in which these muscle jerks appeared earlier or with a longer duration than those in which muscle jerks appeared later or with a shorter duration.

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Table 2 Number of retreat attempts, gasps and escape attempts by treatments (70% N_2 and 30% CO_2 by volume in atmospheric air [70N30C], 85% N_2 and 15% CO_2 by volume in atmospheric air [85N15C], and 90% argon in atmospheric air [AR]) and trials.

Variable	Gas	Category	Number	Trial 1	Trial 2
Retreat attempts (%)	70N30C	1	No retreats	40	44
		2	1 retreat	40	42
		3	2 or more	20	14
	85N15C	1	No retreats	28	50
		2	1 retreat	33	33
		3	2 or more	39	17
	AR	1	No retreats	34	56
		2	1 retreat	54	24
		3	2 or more	12	20
Gasps (%)	70N30C	1	No retreats	37	39
		2	1 retreat	40	47
		3	2 or more	23	14
	85N15C	1	No retreats	34	39
		2	1 retreat	58	42
		3	2 or more	8	19
	AR	1	No retreats	51	64
		2	1 retreat	37	31
		3	2 or more	12	6
Escape attempts (%)	70N30C	1	No retreats	85	81
		2	1 retreat	9	11
		3	2 or more	6	8
	85N15C	1	No retreats	75	94
		2	1 retreat	22	3
		3	2 or more	3	3
	AR	1	No retreats	94	97
		2	1 retreat	3	0
		3	2 or more	3	3

Trial 2

TCREC was higher (P = 0.0206) in 70N30C than AR. Animals with two or more escape attempts in the previous session showed a lower (P = 0.0333) TCREC than pigs without escape attempts (4.7 [± 1.55] vs 7.5 [± 1.21] min). Pigs with high intensity of muscle jerks in the previous session showed a higher (P < 0.0001) TCREC than animals without muscle jerks (7.7 [± 1.24] vs 4.7 [± 1.26] min; P < 0.0001).

The proportion of pigs that lost their posture, the intensity of muscle jerks and instances of gagging was higher (P < 0.05) in 70N30C than in 85N15C and AR (Tables 4 and 5). Pigs lost their posture earlier (P = 0.0191) during the third exposure than during the second and the intensity of muscle jerks was higher (P = 0.0004) in the third exposure than in the first. The intensity of muscle jerks was higher (P < 0.01) in animals in which these muscle jerks appeared earlier or with a longer duration than animals in which muscle jerks appeared later or with a shorter duration. Pigs that performed two or more escape attempts showed the first retreat attempt earlier (P < 0.05) than pigs performing only one escape attempt.

Differences between trials

The incidence of animals losing their posture was lower (P < 0.05) in the second than in the first trial (Table 4).

Discussion

Although it has been stated that the vertical movement of the cradle descending into atmospheric air induces fear in pigs (EFSA 2004), the lack of increase in TCREC on subse-

Table 3 Mean (+ SEM) time taken to perform the first retreat attempt, gasp, escape attempt, muscle jerks, gagging and duration of the muscle jerks by treatments (70% N_2 and 30% CO_2 by volume in atmospheric air [70N30C], 85% N_2 and 15% CO_2 by volume in atmospheric air [85N15C], and 90% argon in atmospheric air [AR]) and trials.

Variable	Gas	Trial 1	Trial 2
Retreat attempts	70N30C	10.4 (± 2.10)	11.9 (± 2.22)
	85N15C	7.5 (± 1.91)	11.8 (± 2.36)
	AR	10.1 (± 2.00)	12.5 (± 2.73)
Gasps	70N30C	16.0 (± 2.09)	13.8 (± 2.22)
	85N15C	12.4 (± 1.65)	12.6 (± 1.75)
	AR	13.4 (± 2.11)	12.0 (± 2.24)
Escape attempts	70N30C	14.9 (± 3.93)	18.0 (± 1.78)
	85N15C	14.9 (± 3.58)	11.0 (± 3.09)
	AR	6.0 (± 6.15)	7.3 (± 4.64)
Loss of posture	70N30C	28.9 (± 0.95)	28.5 (± 0.98)
	85N15C	30.7 (± 0.98)	31.3 (± 1.25)
	AR	33.5 (± 1.33)	30.0 (± 1.72)
Beginning of muscle jerks	70N30C	31.2 (± 0.83)	29.2 (± 0.64)
	85N15C	32.0 (± 0.85)	29.4 (± 0.89)
	AR	28.7 (± 1.35)	27.1 (± 1.47)
First gagging	70N30C	46.3 (± 1.43)	44.1 (± 1.76)
	85N15C	49.3 (± 1.79)	41.3 (± 3.37)
	AR	44.0 (± 6.40)	43.3 (± 4.32)
Duration of muscle jerks	70N30C	14.6 (± 0.89)	10.0 (± 0.66)
	85N15C	12.7 (± 0.91)	8.3 (± 0.93)
	AR	14.5 (± 1.45)	12.5 (± 1.45)

Table 4 Proportion of pigs that lose their posture for treatments (70% N_2 and 30% CO_2 by volume in atmospheric air [70N30C], 85% N_2 and 15% CO_2 by volume in atmospheric air [85N15C], and 90% argon in atmospheric air [AR]) and trials.

Behaviour	Gas	Trial 1	Trial 2
Retreat attempts	70N30C	10.4 (± 2.10)	11.9 (± 2.22)
	85N15C	7.5 (± 1.91)	11.8 (± 2.36)
	AR	10.1 (± 2.00)	12.5 (± 2.73)
Gasps	70N30C	16.0 (± 2.09)	13.8 (± 2.22)
	85N15C	12.4 (± 1.65)	12.6 (± 1.75)
	AR	13.4 (± 2.11)	12.0 (± 2.24)
Escape attempts	70N30C	14.9 (± 3.93)	18.0 (± 1.78)
	85N15C	14.9 (± 3.58)	11.0 (± 3.09)
	AR	6.0 (± 6.15)	7.3 (± 4.64)
Loss of posture	70N30C	28.9 (± 0.95)	28.5 (± 0.98)
	85N15C	30.7 (± 0.98)	31.3 (± 1.25)
	AR	33.5 (± 1.33)	30.0 (± 1.72)

quent training sessions in both trials, indicates that entering the cradle and being lowered into the pit would not affect the pigs aversely if there was atmospheric air, as described by Velarde *et al* (2007).

When the pit contained the gas treatments, TCREC increased compared with the training sessions, as Velarde *et al* (2007) observed when the pigs were exposed to 70 or 90% CO_2 by volume in the same system. Therefore, the exposure to the gas treatments was more aversive for pigs than the exposure to atmospheric air. Although a different order of exposure was applied in Trials 1 and 2 (Table 1), pigs showed a higher TCREC in both trials when the pit contained 70N30C rather than AR. Velarde *et al* (2007) found that pigs had a higher TCREC when the pit was filled with 90% CO_2 than with 70%. Higher concentrations of CO_2 in the stunning system lead to a higher concentration of CO_2 at the entrance of the cradle. This increase of concentration could be detected by the pigs, stimulating the aversion to the gas mixture and increasing the reluctance to enter. Consequently, although Raj and Gregory (1995) stated that concentrations around 30% CO_2 are less aversive for pigs, they could in fact be more aversive than with the use of only inert gases such as argon.

During exposure to the gas mixtures, 58% of the pigs showed retreat attempts, followed by gasping in 56% of the pigs and escape attempts in 11%. These three reactions are considered signs of aversion (Dodman 1977; Raj & Gregory 1996; Lambooij *et al* 1999). Raj and Gregory (1995) suggested that the inhalation of 90% argon by volume in atmospheric air is not aversive for slaughter pigs. In the present study, the proportion of pigs that showed aversion (escape attempts and gasping) to 90% argon was lower than to the gas mixtures with N_2 and CO_2 (70N30C and 85N15C). Therefore, although it cannot be argued that a lack of aversion exists for AR, the aversion to high concentrations of this gas could be lower than when the mixture contains 15 or 30% CO_2. In addition, in comparison to other studies carried out in the same facility (Velarde *et al* 2007), the first retreat attempt appeared later in pigs exposed to AR, 70N30C and 85N15C (15.1 [± 1.61] s, 11.0 [± 1.12] s, and 11.0 [± 1.29] s, respectively) than in pigs exposed to 70 or 90% CO_2 by volume in atmospheric air (6.9 [± 1.24] s and 9.6 [± 1.20] s, respectively).

During the second exposure to the gas treatments, animals showed a higher number of escape attempts and gasps than in the first, the latter also appearing earlier. That result could indicate a prediction of the aversive stimulus by the animals, with an increase in their excitability after their first experience with the gases. This excitability could then affect the effect of the gas mixtures on the animal. In fact, after the signs of aversion, 74% of pigs lost their posture in the present study, considered by Raj and Gregory (1996) and Raj *et al* (1992) as the first indicator of the onset of unconsciousness. However, this loss of posture also appeared sooner in the second exposure to the gas treatments than in the first. According to Broom (2000), a higher excitability in pigs during transport could produce an increase in the respiratory rhythm and Forslid (1992)

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Table 5 Intensity of muscle jerks and presence of gagging movements for treatments (70% N_2 and 30% CO_2 by volume in atmospheric air [70N30C], 85% N_2 and 15% CO_2 by volume in atmospheric air [85N15C], and 90% argon in atmospheric air [AR]) and trials.

Variable	Gas	Category	Number	Trial 1	Trial 2
Intensity of muscular movements (%)	70N30C	1	No convulsion	9	33
		2	Low	14	17
		3	High	77	50
	85N15C	1	No convulsion	25	72
		2	Low	14	11
		3	High	61	17
	AR	1	No convulsion	74	86
		2	Low	20	6
		3	High	6	8
Gagging (%)	70N30C	1	No gagging	40	64
		2	1 gagging	17	22
		3	2 or more	43	14
	85N15C	1	No gagging	57	89
		2	1 gagging	25	3
		3	2 or more	18	8
	AR	1	No gagging	97	92
		2	1 gagging	3	3
		3	2 or more	0	5

reported that a faster and deeper respiration facilitates the uptake of CO_2 and shortens the induction of unconsciousness. In addition, Velarde *et al* (2007) concluded that the higher excitability of halothane-gene carriers, with a higher presence of escape attempts in comparison to halothane-gene-free pigs, induced an earlier loss of posture. Therefore, changes in the excitability of a pig when entering the crate should be considered when the times of exposure to CO_2-stunning systems are stated. For instance, an improvement in the handling of pigs before entering the stunning system in a commercial abattoir could necessitate the monitoring of possible changes in the efficiency of the system with the preset parameters of time and/or gas concentrations.

When the time of exposure to the gas treatments was reduced to 32 s (Trial 2), it was observed that the proportion of animals that lost their posture was higher when exposed to 70N30C than to 85N15C and AR. As the O_2 concentration was the same for all gas mixtures, the time taken to lose posture depended on the CO_2 concentration. It could be concluded that the higher the CO_2 concentration the shorter the time to lose posture, and therefore the time to lose consciousness. These results are in accordance with the work of Raj *et al* (1997). In addition, comparing the results of Velarde *et al* (2007) with those obtained in the present study, the time to loss of posture was similar when pigs were exposed to the gas mixtures used in the present study (33 s) and a 70% CO_2 (34 s), but longer than when a 90% CO_2 by volume in atmospheric air was used (22 s). In fact, the inhalation of a high concentration of carbon dioxide results in rapid increases of carbon dioxide in the blood (Raj *et al* 1997). Consequently, in addition to the effect of a lack of oxygen in the brain (anoxia), the presence of carbon dioxide induces unconsciousness by reducing the pH of cerebrospinal fluid from 7.4 to 6.8 (Raj 1999), and the higher the CO_2 concentration the quicker the effects of hypercapnia on the brain of the pig.

Fifty percent of the pigs in the present study exhibited muscle jerks. From these animals, 41% showed them before loss of posture, 39% at the time of loss of posture and 20% after the loss of posture. In accordance with Raj and Gregory (1996), the muscle jerks before or at the same time as the loss of posture are 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). However, Rodríguez *et al* (2008) stated that animals could be conscious during these movements independently of the loss of posture. Therefore, in the present study, these muscle jerks were studied regardless of the loss of posture and were considered neither as voluntary escape attempts nor as involuntary convulsions, but only as a muscular excitation that occurred before, at the same time or after the loss of posture.

Raj (1999) described muscle jerks as taking longer when pigs were exposed either to argon or to a mixture of argon and CO_2 than when they were exposed to a high concentration of CO_2. In the present study, the presence (percentage of animals that showed convulsions and intensity) was higher in mixtures of CO_2 and N_2 than when argon was used. As these muscle jerks usually occurred at the same time or just after the loss of posture (59%), these results are in accordance with those obtained with other indicators, in which it was observed that 90% AR needed more time to induce unconsciousness than mixtures of N_2 and CO_2.

It was found that TCREC was lower in pigs without muscle jerks in the previous sessions than pigs with a high intensity of these muscle jerks. These muscle jerks could induce traumatism and pain in some animals due to the blows within the cradle. In fact, one pig was replaced in the first trial due to lameness. Therefore, pigs might have associated the pain after or during the muscle jerks with the stunning system and refused to enter the cradle in the following session. In fact, an important increase was observed in TCREC after 3 sessions of gas treatment in both trials, also increasing the number of animals that needed to be pushed into the cradle (Figure 1). Considering that the order of application of the gas treatments was different in each trial, it is suspected that after three sessions the animals increased their reluctance to enter the cradle independently of the gas treatment applied due to the blows with the cradle. However, this reluctance was higher in 70N30C than in the other gas treatments, in this case being difficult to explain by the cause of traumatism in previous sessions in both independent trials. The same was observed for retreat and escape attempts into the cradle. Although an effect of the previous experiences could affect this behaviour, the reaction of pigs was lower when 90AR was applied than in the case of CO_2 treatments.

Animal welfare implications

Nowadays, pigs are stunned with high CO_2 concentrations. As these conditions can be aversive for pigs, gas mixtures with lower CO_2 concentrations, such as 70N30C and 85N15C or high concentrations of inert gases, such as argon, could present good alternatives. However, the lower the CO_2 concentration, the lower the aversion of animals to the gas exposure. Therefore, from an animal welfare point of view, 90% of argon by volume or the lowest possible CO_2 concentrations to stun pigs is recommended, selecting those better suited to particular commercial stunning facilities to ensure the unconsciousness of pigs from stunning until death.

Conclusion

The lack of increase in TCREC on subsequent training sessions indicates that entering the cradle and being lowered into the pit would not cause aversion in the pigs if the pit contained atmospheric air. However, based on the same parameter, it was concluded that the exposure to the gas treatments was more aversive for pigs than the exposure to atmospheric air. On the other hand, the higher the CO_2 concentration was in this study, the higher the aversion was to the gas mixture shown by pigs in terms of escape, retreat attempts and gasping. Therefore, although aversion behaviour was observed in pigs exposed to 90% argon by volume in atmospheric air, this aversion was lower than during the exposure to gas mixtures with N_2 and 15 or 30% CO_2 by volume in atmospheric air. During the second exposure to the gases, a higher prevalence of aversion behaviour was observed and this also occurred earlier. That result could indicate a prediction of the aversive stimulus by the animals. As a consequence, the loss of posture also occurred earlier during the second exposure to the gases than the first. In addition, it was observed that in the present study the higher the CO_2 concentration the shorter the time to loss of posture.

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