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Stunning pigs with different gas mixtures: gas stability

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Abstract

The stability and uniformity of the following gas mixtures: 90% argon; 85% argon and 15% carbon dioxide (CO_2); 70% argon and 30% CO_2; 98% nitrogen (N_2); 92% N_2 and 8% CO_2; 90% N_2 and 10% CO_2; 85% N_2 and 15% CO_2; 80% N_2 and 20% CO_2; 70% N_2 and 30% CO_2; and 90% CO_2 by volume in atmospheric air were assessed in a commercial dip-lift stunning system when the cradle was either stationary or in motion. The gas mixtures of 90% argon, 85% argon and 15% CO_2, 70% argon and 30% CO_2, 85% N_2 and 15% CO_2, 80% N_2 and 20% CO_2, 70% N_2 and 30% CO_2 and 90% CO_2 by volume in atmospheric air could be sustained in a commercial dip-lift stunning system. The stability of the gas mixtures 92% N_2 and 8% CO_2, and 90% N_2 and 10% CO_2 by volume in atmospheric air were lower than in the previous cases. On the other hand, an N_2 concentration higher than 94% by volume in atmospheric air could not be sustained in the stunning system. In addition, gas mixtures of argon and CO_2 showed a higher stability than gas mixtures of N_2 and CO_2. The uniformity at different levels inside the pit (defined as the capacity of the gas to maintain its concentration constant at different levels inside the pit) was higher in 90% argon, or argon and CO_2 mixtures and N_2 and CO_2 mixtures than in 90% CO_2. This fact ensures that for the whole time the animals are inside the pit, the same conditions are applied, which is not the case for 90% CO_2.

Keywords: *animal welfare, argon, carbon dioxide, nitrogen, stability, stunning*

Introduction

Under commercial conditions, two main methods are used to stun pigs (*Sus scrofa*), electrical stunning and carbon dioxide (CO_2). Raj *et al* (1997) and Raj and Gregory (1995) showed argon in air or in association with low concentrations of CO_2 (up to 30% by volume) to be better on animal welfare grounds than high concentrations of carbon dioxide. The relative density of argon (1.38) is higher than air and could therefore be sustained within a pit, as with CO_2, which has a vapour density of 1.53. However, argon has a low presence in the atmosphere (0.9%) and its availability for commercial stunning practices might be limited. Therefore, the cost of industrial grade argon makes it difficult for the industry to implement these gas mixtures, and there is a need to evaluate the feasibility of making use of alternative gas mixtures, such as nitrogen and carbon dioxide. The presence of nitrogen in the atmosphere is around 79% and might be a more suitable gas to be used for stunning pigs. However, the relative density of nitrogen (0.97) is slightly lower than air and its stability, defined as the capability of the gas to be sustained within the pit without being displaced by oxygen, is uncertain. On the other hand, this stability could be increased when nitrogen and CO_2 are combined. The higher the concentration of nitrogen in a gas mixture with CO_2, the lower the relative density of the mixture and, therefore, the harder it is to displace the oxygen in the pit. However, it is uncertain what the behaviour of a mixture of nitrogen and CO_2 (a lighter and heavier gas than air, respectively) would be in comparison with the use of argon and CO_2 (both heavier gases than air) or high concentrations of nitrogen, CO_2 or argon in a dip-lift stunning unit. In this case, three aspects must be considered: i) the capability of the gas or gas mixture to displace air (especially oxygen) in the pit; ii) the stability of the gas in the pit; and iii) the uniformity of the gas mixture throughout the pit, defined as the capability of the different components of the gas mixture to maintain their concentration constantly at different levels within the pit. In the last case, although in commercial conditions the gas stunner tends to be used continuously, inducing a good uniformity of the gas mixtures throughout the pit, when stoppages or breaks occur, this uniformity could disappear. However, it is uncertain which mixtures would be more sensitive to the loss of this uniformity. For instance, if mixtures of CO_2 and nitrogen were used to create less than 2% volume of

Table 1 Gas treatments used in the study.

Treatment	Gas percentages	Relative density*
90AR	90% argon by volume in atmospheric air	1.24
85AR15C	85% argon and 15% CO_2 by volume in atmospheric air	1.40
70AR30C	70% argon and 30% CO_2 by volume in atmospheric air	1.42
98N	98% nitrogen by volume in atmospheric air	0.97
92N8C	92% nitrogen and 8% CO_2 by volume in atmospheric air	1.01
90N10C	90% nitrogen and 10% CO_2 by volume in atmospheric air	1.02
85N15C	85% nitrogen and 15% CO_2 by volume in atmospheric air	1.05
80N20C	80% nitrogen and 20% CO_2 by volume in atmospheric air	1.08
70N30C	70% nitrogen and 30% CO_2 by volume in atmospheric air	1.13
90C	90% CO_2 by volume in atmospheric air	1.35

* At 27°C and 1 atm (based on EFSA 2004).

oxygen then during prolonged periods of stoppages or breaks, CO_2, being heavier than nitrogen, could descend and accumulate at the bottom of the pit and nitrogen could ascend to the top of the pit and eventually disappear into the atmosphere. This stratification would lead to poor animal welfare during gas stunning and killing. In addition, other aspects, such as the tendency of a gas to move from places of high concentration to those of low, could also affect the uniformity of the gas mixture within the pit.

The objective of the study was to assess the stability and uniformity of the gas mixtures indicated in Table 1 in a commercial dip-lift stunning system when the cradle was either static at the bottom of the pit or when it ascended and descended in the pit.

Materials and methods

This study was carried out in the experimental abattoir at IRTA-Monells, Girona, Spain. The abattoir was equipped with a dip-lift stunning system (Butina Alps, Copenhagen, Denmark) that contained a cradle which measured 195 × 61 × 90 cm (length × width × height). The cradle, designed for a single pig, was provided with an entrance guillotine gate at the end of the raceway and an exit ramp gate at the far end. The floor of the cradle was perforated to facilitate the distribution of the gas inside. On closing the gate, the cradle was lowered to the base of a 260-cm deep well with a volume of 8 m^3.

Stability and uniformity of the gas mixtures

The stability and uniformity of ten gas mixtures were assessed on three different days (Table 1). All the gas mixtures had up to 2% residual oxygen in atmospheric air and were tested during three non-consecutive days with a minimum of three days between each replicate. The study was carried out during seven weeks and each day only one gas treatment was applied. After each session the pit was emptied by means of a pump.

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 by two flowmeters (one for argon and N_2 and another for CO_2) that worked at three bars of pressure. The flow rate was 14–16 Nm^3 per hour when gas mixtures or argon and nitrogen treatments were studied and 8 Nm^3 per hour in the case of 90C. The gas mixtures were prepared using a mixing panel prior to introduction into the pit.

The gas mixture concentrations were monitored by measuring 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). The gases or gas mixtures were continuously supplied until the concentration at 60 cm above the bottom of the pit (the level of the head of the pig when the cradle is on the bottom of the pit) was 2% O_2 by volume for 90AR and 98N, 2% O_2 and 8% CO_2 by volume for 92N8C, 2% O_2 and 10% CO_2 by volume for 90N10C, 2% O_2 and 20% CO_2 by volume for 80N20C, 2% O_2 and 15% CO_2 by volume for 85AR15C and 85N15C, 2% O_2 and 30% CO_2 by volume for 70AR30C and 70N30C and 90% CO_2 by volume for 90C. At that point, the flowmeters were closed and the volume of gases used to fill the pit recorded.

The O_2 and CO_2 concentration was monitored at 0, 60, 110, 160 and 210 cm above the bottom of the pit at intervals of 10 min for 1 h and the stunning system remaining stationary. Afterwards, the pit was filled again with the required gas mixture concentrations. A box (60 × 50 × 100 cm) with a similar volume to a pig of 90 kg (0.30 m^3) was placed in the cradle of the dip-lift to simulate the presence of the animal. Then, the cradle was lowered to the bottom of the pit for 23 s, left there stationary for 180 s and raised for 23 s. Afterwards, the cradle remained stationary for 40 s before beginning a new cycle. During the

Table 2 Mean (± SEM) time taken to fill the pit in minutes and mean (± SEM) volume of gas used in Nm^3 to fill the pit for 90% argon by volume in atmospheric air (90AR), 85% argon and 15% CO_2 by volume in atmospheric air (85AR15C), 70% argon and 30% CO_2 by volume in atmospheric air (70AR30C), 92% N_2 and 8% CO_2 by volume in atmospheric air (92N8C), 90% N_2 and 10% CO_2 by volume in atmospheric air (90N10C), 85% N_2 and 15% CO_2 by volume in atmospheric air (85N15C), 80% N_2 and 20% CO_2 by volume in atmospheric air (80N20C), 70% N_2 and 30% CO_2 by volume in atmospheric air (70N30C) and 90% CO_2 by volume in atmospheric air (90C).

Treatment	Time taken to fill the pit (min)	Volume of gas used (Nm^3)			
		Argon	N_2	CO_2	Total
90AR	36.3 (± 4.02)^a	10.0 (± 0.66)	Not used	Not used	10.0 (± 0.66)^b
85AR15C	37.3 (± 4.02)^a	9.6 (± 0.32)	Not used	1.9 (± 0.08)	11.5 (± 0.30)^b
70AR30C	40.3 (± 4.02)^{ab}	7.8 (± 0.17)	Not used	3.3 (± 0.21)	11.1 (± 0.37)^b
92N8C	53.3 (± 4.02)^c	Not used	9.9 (± 0.08)	1.1 (± 0.09)	11.0 (± 0.18)^b
90N10C	52.0 (± 4.02)^c	Not used	10.4 (± 0.40)	1.5 (± 0.12)	11.9 (± 0.28)^b
85N15C	44.0 (± 4.02)^{abc}	Not used	7.5 (± 0.36)	2.0 (± 0.03)	9.5 (± 0.41)^b
80N20C	47.7 (± 4.02)^{bc}	Not used	9.2 (± 0.70)	2.3 (± 0.20)	11.5 (± 0.85)^b
70N30C	45.3 (± 3.48)^{abc}	Not used	6.6 (± 0.68)	3.6 (± 0.23)	10.2 (± 0.50)^b
90C	44.0 (± 4.02)^{abc}	Not used	Not used	4.9 (± 0.03)	4.9 (± 0.03)^a

Means within columns with different superscripts differ significantly ($P < 0.05$).

cycle, the concentration of O_2 and CO_2 by volume at 60 cm was monitored at intervals of 10 s from the beginning of the cycle (10 s before being lowered in the cradle) until the cradle was in the initial position. The monitoring was performed at 60 cm (the level of the pig's head when the cradle was at the bottom of the pit) and at 210 cm from the bottom of the pit. The cycle was repeated four times consecutively and the pit was not refilled during or between cycles. After the four cycles, the O_2 and CO_2 concentration by volume at 0, 60, 110, 160 and 210 cm above the bottom of the pit was monitored at intervals of 60 s for 10 min.

Statistical analysis

Analyses were carried out with the Statistical Analysis System (SAS software, SAS Institute Inc, Cary, NC, USA; 1999-2001). Differences between the gas mixtures, monitoring levels inside each gas treatment when the cradle was stationary, and between cycles when the cradle ascended and descended into the well were analysed with general models (PROC GENMOD). In all cases, a Poisson or negative binomial distribution was applied (Cameron & Trivedi 1998). The residual maximum likelihood was used as a method of estimation. The least square means of fixed effects (LSMEANS) were used when the analysis of variance indicated differences. Differences between the gas mixtures on the total gas used (N_2, argon and CO_2) and the time taken to fill the pit were analysed with the PROC MIXED procedure. When the analysis of variance indicated 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. In all cases, significance was fixed at $P < 0.05$.

Results

Gas and time needed

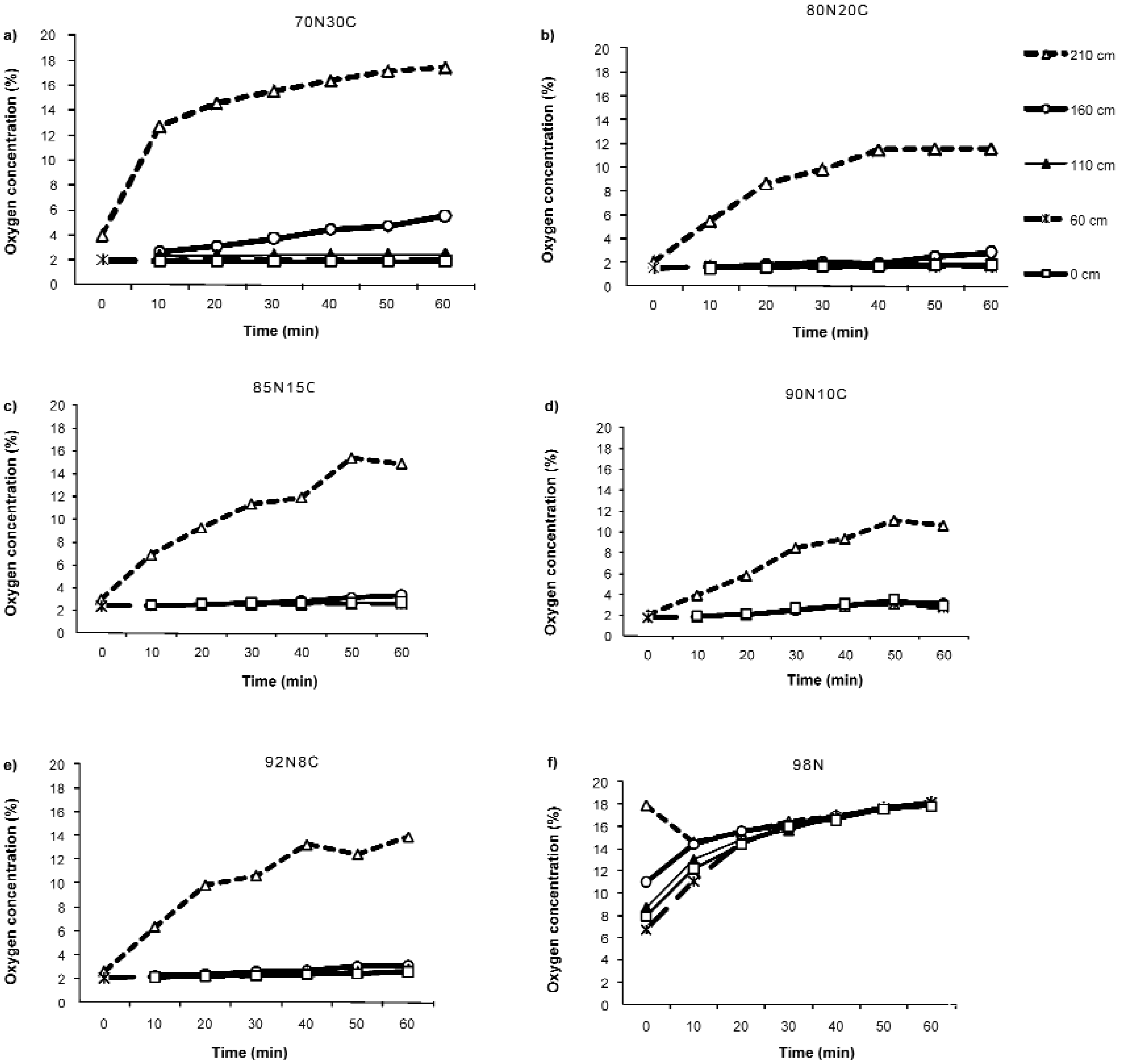
The time taken to achieve less than 2% by volume of oxygen at 60 cm above the bottom of the pit and the volume of CO_2, N_2 and argon used in the different gas mixtures is presented in Table 2. When the pit was only supplied with N_2 (to achieve 98N), the minimal O_2 concentration by volume monitored 60 cm above the bottom of the pit was 6% after a mean time of 90 min. As a result, 98N was not included in the study of stability of the gases. The time taken to fill the pit and the volume of gas components used were affected by the gas mixture ($P < 0.0001$ and $P = 0.0007$, respectively). In most cases, the time taken was around 45 min (Table 2). However, this time was less in 90AR, 85AR15C and 70AR30C than in 92N8C and 90N10C ($P < 0.05$). On the other hand, the volume of gas used with 90C was lower ($P < 0.001$) than with the other gas treatments (Table 2).

Stability and uniformity of the gas mixtures when the cradle was stationary

The O_2 concentration by volume measured at 0, 60, 110, 160 and 210 cm above the bottom of the pit, while the pit was filled and 1 h later, is presented in Figures 1 and 2, respectively.

The O_2 concentration by volume was higher ($P < 0.05$) at 210 cm above the bottom of the pit compared to the other levels in 70AR30C, 70N30C, 80N20C, 85N15C, 90AR, 92N8C and 90C. After 1 h, differences between levels in O_2 concentration by volume were found for the gas mixtures 70AR30C, 85N15C, 92N8C, 70N30C and 90C.

Figure 1



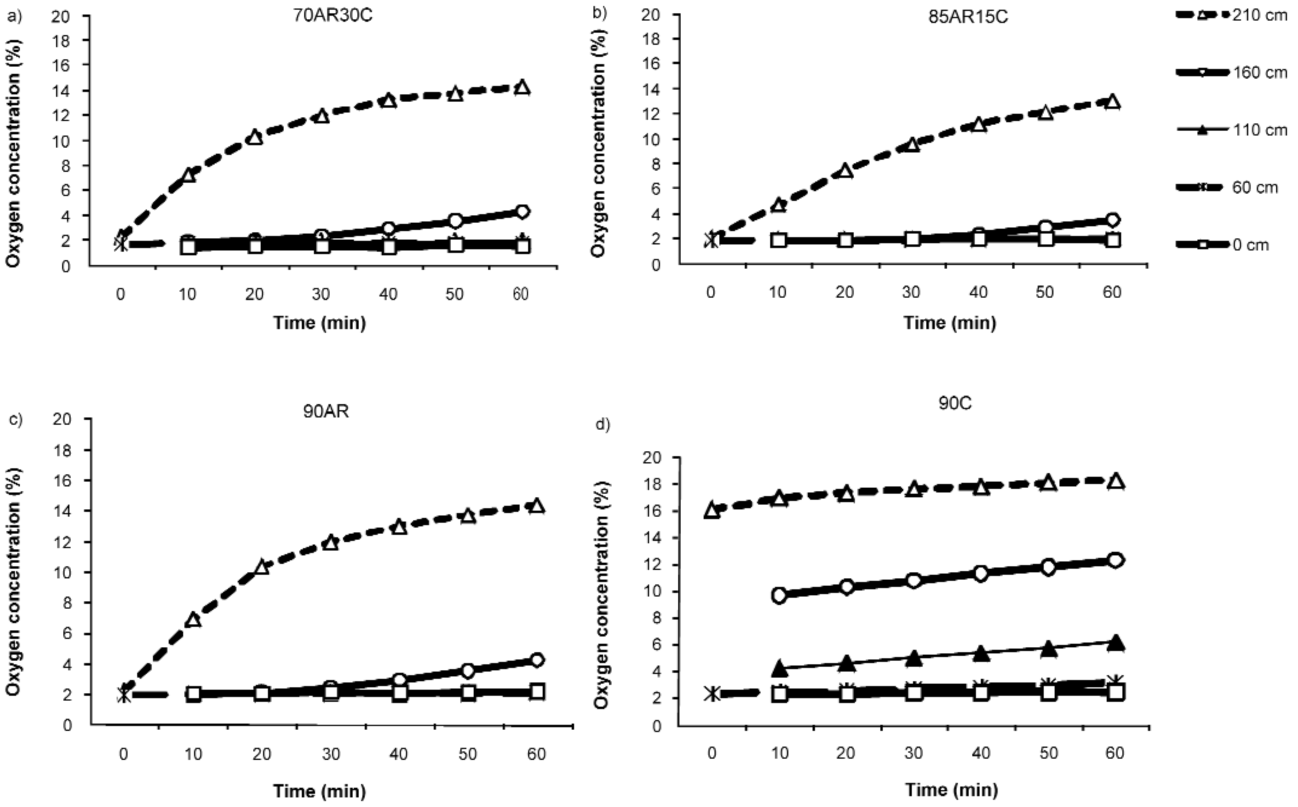
Oxygen concentration by volume at 0, 60, 110, 160 and 210 cm above the bottom of the pit when the cradle remained stationary for (a) 70% N_2 and 30% CO_2 by volume in atmospheric air (70N30C), b) 80% N_2 and 20% CO_2 by volume in atmospheric air (80N20C), (c) 85% N_2 and 15% CO_2 by volume in atmospheric air (85N15C), (d) 90% N_2 and 10% CO_2 by volume in atmospheric air (90N10C), (e) 92% N_2 and 8% CO_2 by volume in atmospheric air (92N8C) and (f) 98% N_2 by volume in atmospheric air (98N).

In the case of CO_2, after filling the pit, differences were found between levels for 70AR30C, 70N30C, 80N20C, 90N10C and 90C. After 1 h, differences between levels in CO_2 concentration by volume were found for 80N20C, 85AR15C, 70AR30C, 70N30C, 85N15C and 90C.

Comparison between gas mixtures

After an hour, with the cradle remaining stationary, the increase of O_2 concentration 60 cm above the bottom of the pit did not differ ($P > 0.05$) between gas mixtures (Table 3). On the other hand, CO_2 concentration at 60 cm above the

Figure 2



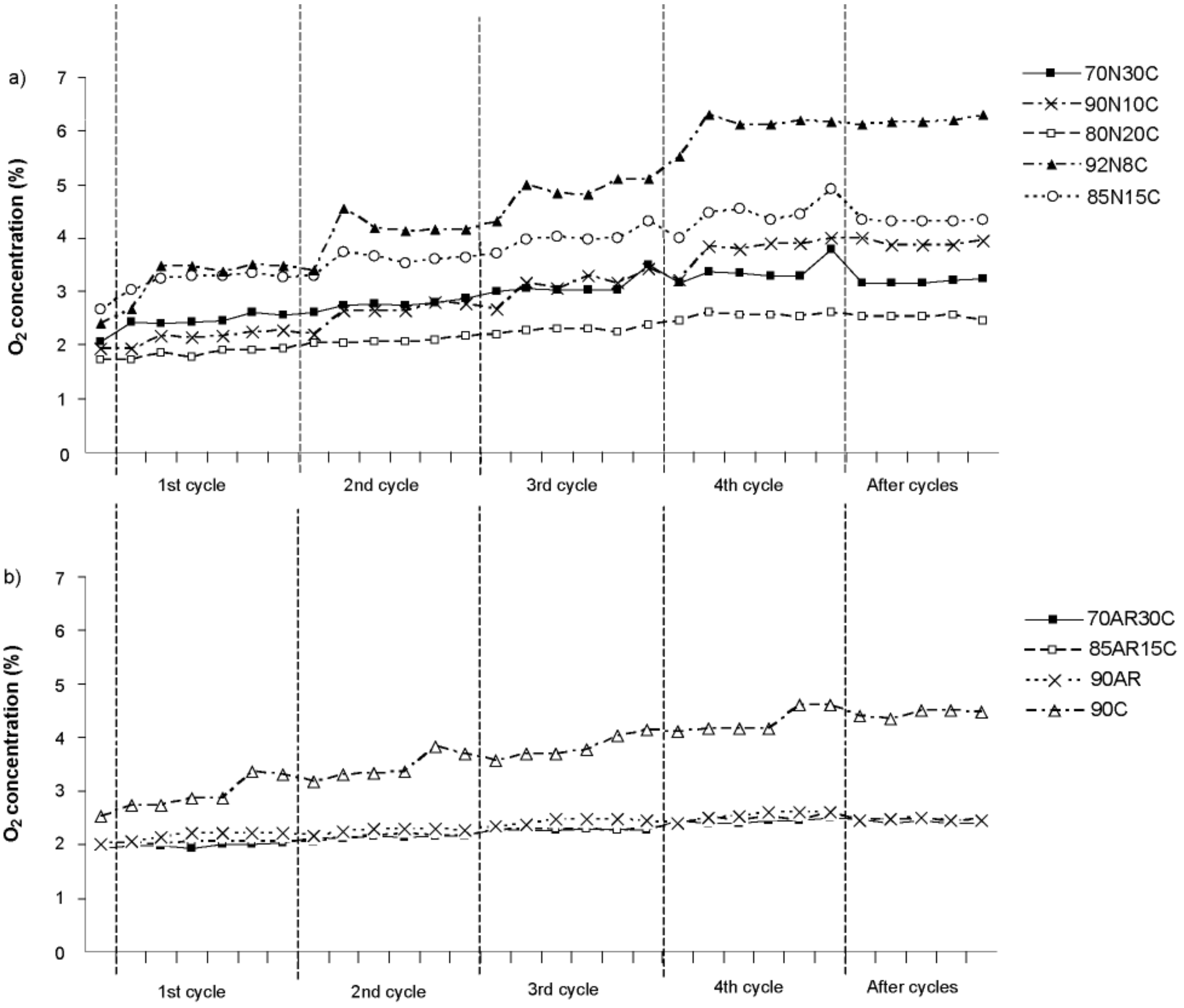
Oxygen concentration at 0, 60, 110, 160 and 210 cm above the bottom of the pit when the cradle remained stationary for (a) 70% argon and 30% CO_2 by volume in atmospheric air (70AR30C), (b) 85% argon and 15% CO_2 by volume in atmospheric air (85AR15C), (c) 90% argon by volume in atmospheric air (90AR) and (d) 90% CO_2 by volume in atmospheric air (90C).

Table 3 **Mean (± SEM) variation in oxygen (O_2), carbon dioxide (CO_2) and nitrogen/argon at 60 and 210 cm from the bottom of the pit after 1 h with the cradle stationary for 90% argon by volume in atmospheric air (90AR), 85% argon and 15% CO_2 by volume in atmospheric air (85AR15C), 70% argon and 30% CO_2 by volume in atmospheric air (70AR30C), 92% N_2 and 8% CO_2 by volume in atmospheric air (92N8C), 90% N_2 and 10% CO_2 by volume in atmospheric air (90N10C), 85% N_2 and 15% CO_2 by volume in atmospheric air (85N15C), 80% N_2 and 20% CO_2 by volume in atmospheric air (80N20C), 70% N_2 and 30% CO_2 by volume in atmospheric air (70N30C) and 90% CO_2 by volume in atmospheric air (90C).**

Treatment	O_2 60 cm	CO_2 60 cm	N_2/Argon 60 cm	O_2 210 cm	CO_2 210 cm	N_2/Argon 210 cm
90AR	+0.1 (± 0.00)	–	–0.1 (± 0.00)^{b}	+7.4 (± 0.13)^{abc}	–	–7.4 (± 0.15)^{ad}
85AR15C	+0.1 (± 0.00)	–0.0 (± 0.03)^{b}	–0.1 (± 0.03)^{b}	+8.4 (± 0.63)^{abc}	–6.0 (± 0.22)^{bd}	–2.4 (± 0.40)^{bce}
70AR30C	+0.0 (± 0.00)	–0.5 (± 0.45)^{b}	+0.5 (± 0.45)^{b}	+7.0 (± 0.26)^{bc}	–9.4 (± 0.44)^{ab}	+2.4 (± 0.38)^{bce}
92N8C	+0.5 (± 0.13)	–0.1 (± 0.00)^{b}	–0.4 (± 0.13)^{b}	+11.3 (± 1.35)^{acd}	–3.7 (± 0.19)^{cd}	–7.6 (± 1.17)^{a}
90N10C	+0.9 (± 0.71)	–0.4 (± 0.20)^{b}	–0.6 (± 0.43)^{ab}	+6.7 (± 0.21)^{bd}	–3.0 (± 0.19)^{d}	–3.7 (± 0.22)^{bd}
85N15C	+0.2 (± 0.06)	–0.1 (± 0.06)^{b}	–0.1 (± 0.03)^{b}	+11.9 (± 0.40)^{ac}	–9.1 (± 0.74)^{b}	–2.8 (± 0.46)^{bc}
80N20C	+0.1 (± 0.06)	–0.2 (± 0.07)^{b}	+0.1 (± 0.03)^{b}	+6.1 (± 1.33)^{b}	–5.5 (± 1.08)^{bd}	–0.6 (± 0.30)^{c}
70N30C	+0.1 (± 0.03)	–0.4 (± 0.27)^{b}	+0.3 (± 0.25)^{b}	+12.4 (± 2.39)^{a}	–15.3 (± 2.67)^{a}	+3.3 (± 1.53)^{b}
90C	+0.8 (± 0.15)	–3.7 (± 0.80)^{b}	+2.9 (± 0.65)^{a}	+1.3 (± 0.41)^{e}	–6.7 (± 2.10)^{bc}	+5.4 (± 1.69)^{ade}

Means within columns with different superscripts differ significantly ($P < 0.05$).

Figure 3



Oxygen concentration by volume at 60 cm above the bottom of the pit during four consecutive cycles and 10 min after the last cycle for (a) 70% N_2 and 30% CO_2 by volume in atmospheric air (70N30C), 80% N_2 and 20% CO_2 by volume in atmospheric air (80N20C), 85% N_2 and 15% CO_2 by volume in atmospheric air (85N15C), 90% N_2 and 10% CO_2 by volume in atmospheric air (90N10C), 92% N_2 and 8% CO_2 by volume in atmospheric air (92N8C) and (b) 70% argon and 30% CO_2 by volume in atmospheric air (70AR30C), 85% argon and 15% CO_2 by volume in atmospheric air (85AR15C), 90% argon by volume in atmospheric air (90AR) and 90% CO_2 by volume in atmospheric air (90C).

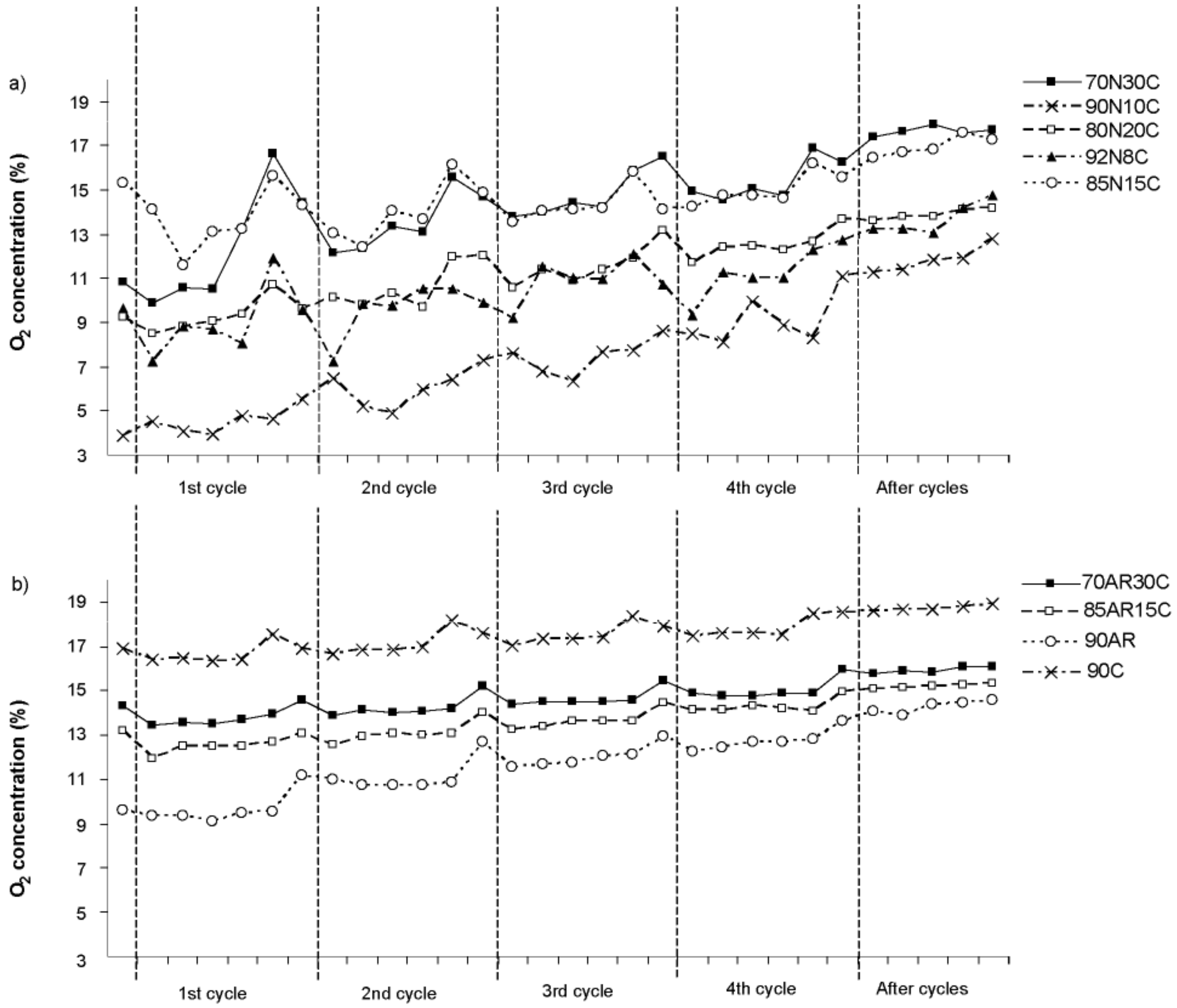
bottom of the pit decreased ($P < 0.0001$) more in 90C than in the rest of the gas mixtures (Table 3).

At 210 cm above the bottom of the pit (the highest point inside the pit), the highest increase of O_2 concentration by volume and decrease of CO_2 concentration by volume was observed in 70N30C ($P < 0.05$; Table 3). The lowest increase ($P < 0.05$) of O_2 by volume was observed in 90C and the lowest decrease ($P < 0.05$) of CO_2 by volume in 90C, 90N10C and 92N8C.

Stability of the gas mixtures when the cradle was not stationary

When the cradle descended and ascended in the pit for four consecutive cycles, the O_2 concentration by volume increased and the CO_2 concentration by volume decreased, both at 60 and 210 cm. At both levels, the increases of O_2 and decreases of CO_2 were different among gas mixtures ($P < 0.05$). However, both O_2 concentration by volume increases and CO_2 concentration by volume decreases were

Figure 4



Oxygen concentration by volume at 210 cm above the bottom of the pit during four consecutive cycles and 10 min after the last cycle for (a) 70% N_2 and 30% CO_2 by volume in atmospheric air (70N30C), 80% N_2 and 20% CO_2 by volume in atmospheric air (80N20C), 85% N_2 and 15% CO_2 by volume in atmospheric air (85N15C), 90% N_2 and 10% CO_2 by volume in atmospheric air (90N10C), 92% N_2 and 8% CO_2 by volume in atmospheric air (92N8C) and (b) 70% argon and 30% CO_2 by volume in atmospheric air (70AR30C), 85% argon and 15% CO_2 by volume in atmospheric air (85AR15C), 90% argon by volume in atmospheric air (90AR) and 90% CO_2 by volume in atmospheric air (90C).

not different between cycles ($P > 0.05$) and no interaction was found between cycle and gas treatment ($P > 0.05$).

At 60 cm, the O_2 concentration by volume increased after each cycle, (Figure 3). This increase was lower ($P < 0.05$) in 90AR, 85AR15C, 70AR30C and 80N20C in comparison to 92N8C. At 210 cm, the O_2 concentration by volume increased after each cycle (Figure 4), this increase being higher ($P < 0.01$) in 92N8C and 70N30C than in 90AR, 85AR15C, 70AR30C, 90N10C, 80N20C and 90C, and in 85N15C than in 85AR15C, 70AR30C, 90N10C and 90C ($P < 0.05$).

At 60 cm, the CO_2 concentration by volume decreased after each cycle. This decrease was greater ($P < 0.001$) in 90C in comparison to all the other gas mixtures. At 210 cm, the CO_2 concentration by volume decreased after each cycle, this decrease being greater ($P < 0.05$) in 90C than in the other gas mixtures and in 70N30C than in 85AR15C, 70AR30C, 92N8C, 90N10C and 80N20C ($P < 0.05$). This decrease was also greater ($P < 0.05$) in 70AR30C, 80N20C and 85N15C than in 85AR15C and 90N10C.

Discussion

The mean time taken to fill the pit of 8 m^3 with a flow rate of 14–16 Nm^3 per hour was around 45 min when the gas mixtures contained between 70 to 85% of N_2 and 30 to 15% of CO_2. The time was shorter when the pit was filled with argon mixtures and 90% argon by volume and longer when the N_2 concentration of the gas mixtures was higher than 85% by volume in atmospheric air.

When the pit was supplied with only N_2 in order to obtain 98% N_2 and 2% of residual O_2 by volume in atmospheric air, after 90 min, the minimal O_2 concentration by volume achieved 60 cm above the bottom of the pit was 6%. The lower relative density of the N_2 compared to the atmospheric air prevented the stability of this gas within a well, with a residual O_2 concentration lower than 2% by volume. This result indicates that in the current commercial dip-lift stunning systems, the stunning of pigs with hypercapnia induced by CO_2 cannot be replaced by stunning with anoxia induced with the inhalation of only N_2.

The volume of gases used in all gas mixtures (around 10–12 Nm^3) was higher than the volume of the well (8 m^3), except in 90C, where the volume of CO_2 used was 4.9 (± 0.03) Nm^3. Consequently, the CO_2 used in the 90C treatment was insufficient to fill the pit, but following the protocol established for all the gas treatments, the flowmeter was closed at a mean time of 44.0 (± 4.02) min. The reason for this was that we fixed as an objective to have less than 2% oxygen by volume at 60 cm from the bottom of the pit (the level of the head of the pig when the cradle is at the bottom of the pit) to consider the pit filled enough with any of the gas treatments tested. For all gas mixtures, except 90C, below 160 cm, the O_2 concentration was lower than 2% by volume. In fact, the gas mixture 90C had gas stratification and an O_2 concentration below 2% was only observed between 0 and 60 cm above the bottom of the pit (see Figures 1 and 2). Two facts could explain this effect, not being mutually exclusive.

Firstly, the two flowmeters used to mix the gases before entering the stunning system had a different flow rate. Actually, to ensure a mean flow rate for all gas treatments of 14–16 Nm^3 per hour, a flowmeter was used that could supply N_2 and argon at a maximum flow rate of 20 Nm^3 per hour. However, as the concentration of CO_2 ranged from 8 to 30% by volume in the gas mixtures, a smaller flowmeter was necessary for CO_2, allowing a better control of low flow rates, such as 2–3 Nm^3 per hour. Consequently, the maximum flow rate for the flowmeter used for CO_2 was 8 Nm^3 per hour. This lower flow rate could have affected the 90C treatment that was, in comparison, the only treatment that could not be filled at 14–16 Nm^3 per hour. Therefore, the slower supply of CO_2 in the system could explain why it took the same time as the rest of the treatments to achieve < 2% of oxygen at 60 cm from the bottom of the pit, but using only 50% of the gas used in the other treatments.

The second fact could be the higher relative density of CO_2 compared with atmospheric air. After 1 h in stationary conditions, all the gas mixtures, except 90C, showed small changes in their concentrations at 60 cm from the bottom of the pit. However, in 90C, the CO_2 concentration by volume increased at this level, dropping from upper levels.

This tendency of CO_2 to go down into the pit was also confirmed by the fact that at 210 cm from the bottom of the pit the higher increase of O_2 after 1 h in stationary conditions was for the treatment 70N30C (one of the mixtures with a higher CO_2 concentration), in which CO_2 tended to drop to lower altitudes in the pit. However, this tendency of CO_2 to descend was not so clear when heavier gases than N_2, such as argon, were combined with CO_2. In fact, the effect found for 70N30C, in which at the lower levels CO_2 substituted N_2 and at the highest levels N_2 substituted CO_2, was not observed in the case of 70AR30C, probably due to them having similar relative density. Therefore, the uniformity, defined as the capacity of the different components of the gas mixture to maintain their concentration constant at different levels inside the pit, decreased as the CO_2 concentration of the gas mixture increased. At the same time, the uniformity was higher in gas mixtures of argon and CO_2 than in gas mixtures of nitrogen and CO_2.

The gas mixtures with higher stability would be those with the lowest increase of O_2 and/or decrease of CO_2 at 60 and 210 cm above the bottom of the pit when the cradle is static or in motion through the pit. When static, the increase of O_2 by volume at 60 cm was not significantly different between gas mixtures. However, the decrease of CO_2 concentration was higher in 90C than in all the other gas mixtures. In this case, the similarity in O_2 concentrations in 90C is due to the descent of the CO_2 to the bottom of the pit, being replaced mainly by the nitrogen of the atmospheric air (as it contains 79% of N_2 and only 20% of O_2). When the cradle was in motion, and according to the O_2 increase at 60 cm above the bottom of the pit, the best stability was found in 90AR, 85AR15C, 70AR30C and 80N20C, and the worst in 92N8C. At the same time, according to the decrease of CO_2 when the cradle was in motion, 90C was the worst gas treatment.

On the other hand, when O_2 gains or CO_2 losses are monitored at the highest level (210 cm from the bottom of the pit) 90C must be considered separately due to the high stratification observed from the beginning, just after filling the pit (Figure 1). In fact, at this moment, it was the treatment with the highest O_2 concentration by volume at this level (16%) and more similar to the atmospheric one (20%), and as a consequence it had the lowest O_2 concentration increase after 1 h motionless and after the four cycles. On the other hand, the gas treatments that showed the highest O_2 concentration increases at 210 cm were 70N30C, where O_2 from atmospheric air replaced the CO_2 that descended to the bottom of the pit, and 92N8C, where the O_2 from the atmospheric air replaced the N_2 that had left the system. In addition, at the same CO_2 concentration in a gas mixture, the loss of CO_2 or the increase of O_2 at 210 cm was lower when the gas treatment contained argon rather than N_2.

Currently, in most commercial pig-stunning systems, the time of exposure to the maximum concentration of gases has to be considered from the moment the cradle reaches the bottom of the pit, due to the different concentrations of CO_2 at different levels (EFSA 2004). If alternative gas mixtures are used in current pig-stunning facilities, their stability could be a concern. However, gas mixtures of argon and CO_2, 90% argon, or mixtures of N_2 and CO_2 with a nitrogen concentration similar to the atmospheric one (79%), have high stability and high uniformity, avoiding the need to lower the animal to the bottom of the pit to be exposed to the gas treatment. Therefore, although it has been stated that a longer time is needed to stun pigs with mixtures of argon and CO_2 and N_2 and CO_2 than with 90C (Raj 1999), it is also true that, given the same facilities, with these gas mixtures animals could be exposed longer to anoxic conditions (< 2% residual oxygen by volume) than using 90C. This fact could have advantages from an animal welfare point of view, as in the more modern stunning systems, in which animals are stunned in groups of 6 to 10 animals, a first stop for the pigs in the pit exists in which CO_2 is high enough to produce aversion but too low to produce unconsciousness rapidly. The results obtained in the present study show with the use of gas mixtures, the conditions at the first stop could be similar to those found at the bottom of the pit, ensuring the desired treatment to the animal from the first moment.

Animal welfare implications

Nowadays, pigs are stunned with high concentrations (70–90%) of CO_2 (Velarde *et al* 2000). However, this gas, above 30% by volume in atmospheric air, causes aversion in pigs (Raj & Gregory 1996). Alternative gases to CO_2, producing an anoxic state in the animal when residual O_2 is below 2% by volume, are being considered. However, their stability in commercial stunning systems differs as a result of the different relative densities of the gases. According to our results, concentrations of N_2 cannot be sustained at a higher concentration than 94% by volume. Therefore, this gas must be mixed with other gases to stun pigs. In terms of stability, mixtures of N_2 or argon and CO_2 up to 30 and 90% argon by volume in atmospheric air could be used to reduce the aversion of pigs to high CO_2 concentrations. In comparison to 90% CO_2, the use of these gas mixtures or argon would increase the time animals are exposed to the desired concentrations due to a higher uniformity of the gas treatments inside the pit. This fact ensures that all the time the animal is inside the pit, the same conditions are applied; this not being the case for CO_2 where it may well be that high variability is seen between slaughterhouses.

Conclusion

Only with N_2, could the O_2 concentration inside the pit not be reduced below 6%. This O_2 concentration is too high to induce unconsciousness by anoxia in pigs. Therefore, in commercial stunning systems, the N_2 should be mixed with other gases, such as CO_2, to stun pigs. The results indicated that argon alone or argon and CO_2 mixtures are better than nitrogen and CO_2 mixtures in terms of stability and uniformity.

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