Animal 19 (2025) 101573

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Pigs exposed to nitrogen, argon or carbon dioxide filled high-expansion foam: behavioural responses, stun process and blood lactate concentration

C. Lindahl [a,*], E. Sindhøj [a], M.A. Gerritzen [b], H.G.M. Reimert [b], C. Berg [c], M. Blad [a,c], A. Wallenbeck [c]

[a] Department of Agriculture and Environmental Engineering, RISE Research Institutes of Sweden, PO Box 7033, SE-750 07 Uppsala, Sweden
[b] Department, of Animal Health and Welfare, Wageningen University & Research, De Elst 1, PO Box 338, Wageningen, the Netherlands
[c] Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, PO Box 234, SE-532 23 Skara, Sweden

ARTICLE INFO

Article history:
Received 21 August 2024
Revised 30 May 2025
Accepted 3 June 2025
Available online 9 June 2025

Keywords:
Controlled atmosphere stunning
Euthanasia
Gas stunning
Inert gas
Slaughter

ABSTRACT

According to the EU legislation, all animals farmed for food production must be stunned before being exsanguinated (exempt slaughter prescribed by religious rites). Stunning methods must be reliable, effective, and free from avoidable pain, distress, and suffering, warranting continuous improvement. New methods must be thoroughly evaluated from an animal welfare perspective before approval. One technology developed for on-farm euthanasia and large-scale depopulation for disease control in pigs uses high-expansion foam to create an anoxic atmosphere in a closed container. The method has previously been suggested as a potential method for stunning pigs at slaughter. This study compared the behavioural responses and stun process (e.g., loss of posture and convulsions) of pigs exposed to three different gases (N_2, Ar, and CO_2) delivered in high-expansion foam. Thirty-six pigs, approximately 12 weeks old, were placed one at a time in a container and exposed to either N_2, Ar, or CO_2 gas-filled foam for 5 min from foam start. Behavioural observations were conducted from video recordings, assessing time to loss of balance, loss of posture, last strong convulsion and last muscle contraction. Results showed that pigs in the CO_2 treatment performed escape attempts significantly earlier than in N_2 and Ar, and there were more pigs that performed this behaviour in CO_2, indicating that high concentrations of CO_2 are more aversive than Ar and N_2. Pigs exposed to CO_2 foam also avoided the foam earlier compared to the other two gases. Loss of posture occurred earlier in the CO_2 treatment, consistent with the anaesthetic effect of CO_2. A faster foam filling time for CO_2 foam may be a contributing factor to the differences found; however, filling time was adjusted for in the statistical analyses to reduce bias in the comparisons between gases. All pigs across treatments were adequately stunned after 5 min, with no corneal reflex, rhythmic breathing, gagging, or muscle contractions upon removal from the container. No indications of regained consciousness during sticking and bleeding were found. In conclusion, the gas foam method was effective in stunning the pigs regardless of the gas type used. The less aversive responses to Ar and N_2 foam are positive from an animal welfare perspective, but the longer time to loss of consciousness compared to CO_2 is a disadvantage.

Implications

A technology developed for on-farm killing of e.g., seriously injured or diseased pigs has been suggested as a potential stunning method for pigs at slaughter. This method uses gas-filled high-expansion foam to create an anoxic (< 1% oxygen) atmosphere. The behavioural responses of pigs exposed to foam filled with three different gases (N_2, Ar, and CO_2) were evaluated. All pigs, regardless of the gas used, were effectively and adequately stunned and euthanised. The behavioural responses of pigs indicated less aversiveness during exposure to Ar and N_2, compared to CO_2. However, the prolonged time to reach unconsciousness with Ar and N_2, compared to CO_2, is a disadvantage.

Introduction

Stunning and killing methods for pigs at slaughter and for other reasons should be reliable and effective, and free from any avoidable pain, distress, and suffering (EFSA, 2013; European Council (EC) Regulation No. 1099/2009). Current methods have various advantages and disadvantages concerning animal welfare, and no

* Corresponding author.
E-mail address: cecilia.lindahl@ri.se (C. Lindahl).

https://doi.org/10.1016/j.animal.2025.101573
1751-7311/

method meets all desirable qualities (EFSA, 2004; Sindhøj et al., 2021; Wallgren et al., 2021). Controlled atmosphere stunning with high concentrations of carbon dioxide (CO_2) is common in commercial pig slaughter due to its advantages, such as preslaughter handling and group stunning without firm restraint (Atkinson et al., 2020). However, CO_2 stunning is not immediate and causes severe respiratory distress, aversion and discomfort before pigs lose consciousness (Raj and Gregory, 1996; Velarde et al., 2007; Rodríguez et al., 2008; Verhoeven et al., 2016; Atkinson et al., 2020; Terlouw et al., 2021). This has led to calls for alternatives to improve pig welfare at slaughter from consumers, industry, animal rights organisations, academia, authorities, and legislators.

Inert gases like nitrogen (N_2) and argon (Ar) have been proposed as alternatives to CO_2, as they have been shown to cause less aversion (Dalmau et al., 2010b; Llonch et al., 2012b). However, these gases require longer exposure times and may result in pigs regaining consciousness faster (EFSA, 2004), making irreversible stunning crucial. Like CO_2, Ar has a higher density than air and could therefore be used in existing systems for CO_2 stunning (Atkinson et al., 2020), but Ar is more expensive. The N_2 gas is more affordable but poses technical challenges due to its similar density to air, making it difficult to create and maintain an anoxic, controlled atmosphere with current gas–stunning technology (Atkinson et al., 2020; Dalmau et al., 2010a). Improving process engineering to consistently produce and maintain an anoxic (< 2% O_2) atmosphere is a priority (Steiner et al., 2019; Sindhøj et al., 2021).

A new technology for on-farm euthanasia and large-scale depopulation creates an anoxic atmosphere using high-expansion foam to deliver N_2 gas (HEFT, 2023). This method, named NEFS in container (Nitrogen Expansion Foam Stunning in container), achieves anoxic conditions faster and with lower gas consumption than using free N_2 (Lindahl et al., 2020). The European Food Safety Authority's Panel on Animal Health and Welfare has recently delivered a scientific opinion on the use of high-expansion foam for stunning and killing pigs and poultry (EFSA, 2024). The conclusion was that NEFS in container can be suitable for killing purposes other than slaughter for certain categories and types of pigs. Research is needed to better understand the stun process, pig responses to the foam, and the method's reliability for the method to be considered for slaughter purposes. Lindahl et al. (2020) provided proof of principle of the method for stunning weaner pigs, and McKeegan et al. (2013) showed it for poultry, showing that the N_2 foam method quickly achieves and maintains an anoxic atmosphere (< 1% O_2) throughout the stunning process.

Various preslaughter factors, including the stunning process, can cause stress in pigs. Evaluating stress associated with the slaughter process can be achieved by analysing blood parameters, e.g., lactate, from samples taken during exsanguination (Edwards et al., 2010b). During prehandling and stunning, movements requiring considerable muscle activities are performed and such physical stress will lead to an increase in lactate concentration in the blood. Lactate concentration in blood samples taken at exsanguination is an important factor in relation to slaughter, as it has been shown to be related to pork quality (Edwards et al., 2010a; Hambrecht et al., 2004). This study aimed to compare the behavioural responses, stun process (e.g., loss of balance (**LOB**), loss of posture (**LOP**) and convulsions) and blood lactate concentration of 12-week-old pigs exposed to three different gases (N_2, Ar and CO_2) delivered in high-expansion foam.

Material and methods

The experimental studies were conducted at the pig facility of the Swedish Livestock Research Centre, Lövsta, Uppsala, at the Swedish University of Agricultural Sciences over 5 days in September-October 2021.

Two parallel studies were conducted, one focusing on the behavioural responses of the pigs (presented here) and one focusing on the loss of consciousness measured with electroencephalogram (**EEG**). Pigs equipped with EEG electrodes had to be restrained to enable the use of equipment, which significantly affected the pigs' behavioural responses. For the behavioural study, pigs were allowed to move freely in the container, without attached equipment, to observe the behavioural responses to the gas-filled foam stun process. Having two pigs treated in parallel also likely reduced isolation stress, as they could see, hear, and smell each other through a perforated window between containers. A total of 72 pigs were used across both studies. This study focused on the 36 pigs used for behavioural observations.

Pigs

A total of 36 pigs, approximately 12 weeks old (86.5 ± 2.9 days), were used in the experiments. The mean pig BWs and SDs on the day of the experiments were 52.2 ± 5.8 kg, 47.6 ± 9.2 kg and 50.2 ± 6.7 kg for treatments N_2, Ar and CO_2, respectively.

Sample size was determinated based on variation in behaviour and latency variables from a previous study assessing differences in reaction to air-filled and N^2-filled foam (Lindahl et al., 2020). In the current study, the sample size was 12 pigs per treatment compared to 20 pigs per treatment in the previous study. A posthoc sample size calculation indicated that differences between treatment groups on a level of ± 1.1 s for continuous latency variables and 3.1% of binomial behavioural variables would be able to detect.

The pigs were crossbreds from nine different litters, with dams of Yorkshire breed or Yorkshire-Landrace crosses and sires of Hampshire (21 pigs) or Duroc (15 pigs) breed. The pigs were divided into three treatments (n = 12). From each litter, three pigs of each sex were randomised to treatment, ensuring equal numbers of females and immuno-castrated males from each litter represented in respective treatment.

The pigs were born and raised at the pathogen-free pig research facility with integrated pig production (Swedish University of Agricultural Sciences, 2017). They were weaned at 5 weeks of age and remained in the farrowing pens for an additional 5 weeks after weaning before being moved to the growing-finishing pig units.

The experimental equipment

The high expansion foam system is based on controlled atmosphere stunning principles. Animals are placed inside a container which is filled with high-expansion foam to create an anoxic atmosphere. Different gases (N_2, Ar or CO_2) were used to produce the foam bubbles, effectively displacing the ambient air and creating an atmosphere with close to 100% gas and oxygen (O_2) concentrations below 1%. Once the container is completely filled with foam, the foam is destroyed by a burst of gas, allowing visibility to observe the pigs. The anoxic environment is maintained until the container is opened.

Two modified C1 systems from the Swedish company High Expansion Foam Technology (**HEFT**) AB (HEFT, 2022) were used for the study. Each container measured L1200xW800xH910 mm with a floor area of 0.78 m^2 and had two foam generators placed in diagonal inside corners and an external docking station for delivering the gas and diluted foaming agent. A 50-litre bottle with compressed N_2, Ar or CO_2 (200 bar; Linde Gas AB, Uppsala, Sweden), reduced to 7 bar, was connected to the docking station. A solution of water and 5% foam agent (Agrifoam C5, Tyco Fire Protection Products, UK) was used for foam production. The containers had a side door for pigs to enter and a top lid for easy access. Transparent acrylic (Perspex) windows in the floor and lid allowed video recordings from above and from below. Clear adhesive anti-slip tape was applied to the inside floor to prevent pigs from slipping.

The experimental set-up involved two containers attached along one side with a transparent and perforated window between them, allowing the pig in each container to see, hear and smell each other (Fig. 1 a and b). The containers were placed over a shallow pit for camera placement underneath. Flexible LED light strips were mounted inside the containers and below the containers to improve visibility. One video camera (GoPro 7 black, GoPro, San Mateo, California, U.S.) was mounted above and one below the containers to record the pigs' behaviour. A microphone inside the container recorded vocalisations.

Oxygen concentrations inside the containers were continuously monitored using a flow-through fluorescence-based optical oxygen sensor (SST-Sensing, UK) connected to a microcomputer with data logged to an SD card. An air pump connected to a liquid trap and a moisture-absorbing filter sampled the atmosphere at snout height. The sensor also continuously recorded the temperature. The sampling tube inlet was covered with a hydrophobic PTFE membrane with 0.45 μm pores (general product available from multiple manufacturers) to further protect the sensor from moisture.

Experimental procedure

The pigs were weighed on the day of the experiments. All selected pigs from one pen (i.e. litter) were moved to a temporary pen near the experimental setup, where they acclimatised for at least 10 min.

The treatments were conducted in batches of two pigs, one with EEG equipment (restrained) and one with no equipment. Treatments (N_2, Ar and CO_2) were randomised within groups of three consecutive batches with pigs of the same litter and sex; thus, the order of gases within the three consecutive batches varied. The first pig was moved to the container, restrained, and fitted with EEG electrodes. The second pig was then moved to the parallel container. Both pigs were allowed to acclimatise for 1 min before foam production started. The foam was filled with one of the three gases (same gas for both containers): N_2, Ar or CO_2. Once the containers were completely filled with foam, several quick gas pulses destroyed the foam to increase visibility. The entire procedure was video recorded, and oxygen concentrations in the containers were logged continuously.

Five minutes after foam production began, the containers were opened, and the pigs were assessed for signs of consciousness by controlling corneal reflexes (by touching the pig's cornea and checking for any movement of the eyelid (blinking)), muscle contractions (any visible muscle movement) and gagging or breathing. If no reflexes, muscle contractions, gagging or breathing were shown, the pig was considered as being in a state of deep unconsciousness. A stethoscope was used to control for heartbeats (present or not) before sticking and bleeding. The 5-min exposure time was chosen based on the previous study on weaner pigs by Lindahl et al. (2020), where the results showed all pigs were in deep unconsciousness or dead after exposure to N_2–filled foam.

During bleeding, blood lactate analysis was conducted with a hand-held meter (Lactate Plus; Nova Biomedical GmbH, Germany). A small sample of blood was collected with a plastic spoon and a test strip was immediately dipped into the collected blood. The analyser provided the lactate concentration (mM) in approximately 13 s. Blood lactate was measured in only ten pigs per treatment due to practical issues.

The containers were rinsed clean with water between batches.

Behavioural observations

One observer (MB), not involved in the practical experiments, conducted all behavioural observations based on video recordings from above and below the container. The treatments were blinded to the observer.

The pigs' behaviour was assessed according to the definitions in Table 1. Observations began 30 s before and lasted 100 s after foam started. Behaviours were registered as the number of events per 10 s intervals, three intervals before and 10 intervals after foam started. The observation period was set to ensure that LOP for all

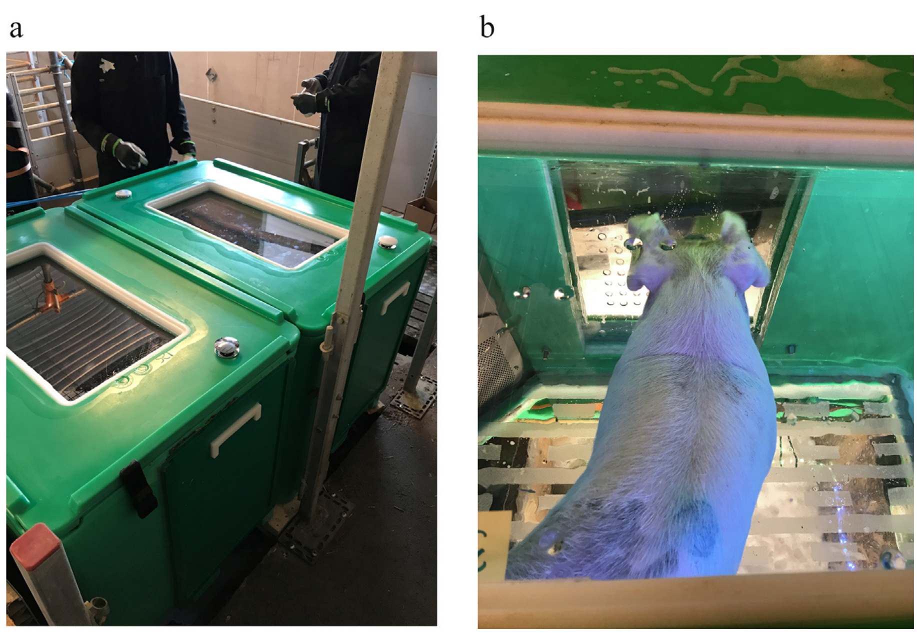

Fig. 1. The experiment setup with (a) two attached containers with a perforated window (b) allowing the pigs to see and smell each other to decrease isolation stress (photo: RISE).

Table 1
Definitions of the pig behaviours included in the behavioural analysis.

Behaviour	Definition
Sit	Stationary, resting on the caudal part of the body with support of front hooves
Lay down	Lying position with body in contact with the floor
Slip	One or more hooves are sliding quickly and uncontrolled on floor
Startle	A sudden, involuntary movement/jump in response to something unexpected
Retreat	Walks in reverse
Shake	Vigorous shake of head and body
Explore floor	Snout in contact with floor
Explore wall	Snout in contact with wall
Explore foam	Snout in contact with foam
Explore window	Snout touches the window
Avoid foam	Pig stretches head above foam when foam level becomes high to avoid getting head and snout under foam
Escape attempt	Jumps up against the lid, head touches lid
Gasping	Deep breath through a wide-open mouth, which may involve stretching of the neck (Dalmau et al., 2010b)
Vocalisation	
Grunt	Low-pitch vocalisation
Squeal	High-pitch vocalisation
Scream	Very loud, strong high-pitch vocalisation
Defecate	Self-explanatory
Loss of balance	Pig swaying, staggering, and falling but struggles to regain its position (righting reflex)
Loss of posture	Pig loses posture, inability to remain standing with no attempts to righten itself
Strong convulsions	Forceful, galloping or kicking, movements with a high frequency
Last muscle contraction	Last visible muscle movement
Gagging	Low-frequency inhalations with opening/closing of mouth and occasional emission of sounds similar to snoring

pigs was included, with all pigs lying on their sides by the end of the period. Observations continued beyond 130 s to register the time to the last visible muscle contraction.

The stun process was described by defining time from foam start to LOB and LOP. After LOP, convulsions (muscular excitation) commonly occurred and were described qualitatively by their intensity and type. The times until last observed strong muscular contraction and last visible contraction were registered. Any gagging observed after LOP was also noted. The definitions of LOB, LOP, strong convulsions, last muscle contraction and gagging are presented in Table 1.

Statistical analyses

Behavioural data were divided into 10 s intervals, with the first three intervals (1–3) occurring before foam production and the next ten intervals (intervals 4–13) during foam production. Behaviour was recorded as counts. When behaviour was analysed over time (intervals), the behaviours were converted to a binary variable (observed or not observed) in each interval. Vocalisations were recorded as counts before and counts after LOP.

Statistical analyses were performed using SAS version 9.4 (SAS, 2021; Cary, NC, USA). Descriptive statistics were calculated using Proc Means, and Proc Freq.

Differences between treatments (N_2, Ar and CO_2) were analysed using multivariable models, and all models developed stepwise forward including tests of all relevant interactions. Continuous outcome variables (blood lactate concentration, foam filling time, latencies for loss of balance, loss of posture, last strong convulsion and last convulsion) were normally distributed (residuals examined for normal distribution using the Proc Univariate, considering Shapiro-Wilks test for normality and a normal probability plot), and analysed with a GLM (Model 1) in Proc GLM. Differences in binomial outcome variables (behaviours performed or not during each 10 s interval) were analysed using generalised linear model (Model 2) in Proc Glimmix (with binomial distribution, logit link).

Model 1: y = Treatment + Sex + Test day + Weight on test day + Foam filling time + e

Model 2: y = Treatment + Interval + Sex + Test day + Weight on test day + Foam filling time + Treatment*Interval + e

Where predictor variables Treatment (N_2, Ar and CO_2), Sex (female or immuno-castrated male), Test day (1–5) and Interval (1–13) were fixed class effects, and Weight on test day and Foam filling time was included as fixed continuous covariates. When foam filling time was assessed as a y-variable, foam filling time was not included in the model.

Additionally, the linear association between latency for LOP and LOB and lactate concentration was tested by adding lactate as a linear regression covariate in Model 1.

Differences between treatments in a total number of pig vocalisations (counts of grunting and screaming) before and after LOP were analysed using Kruskal-Wallis tests, as the skewed distributions of these outcome variables did not allow analyses with a GLM (Model 1) as primarily intended.

Results

Foam filling time, temperature, and oxygen concentration

The container was filled with foam until it started coming out of the overflow valve on the lid. The valve was then closed, and foam production was stopped. The mean filling time was shorter for the CO_2 treatment (LSM ± SE 72.1 ± 4.56 s; $P < 0.001$) compared to the N_2 and Ar treatments (102.1 ± 4.63 s and 114.2 ± 4.65 s, respectively). To determine if excessive convulsions in the N_2 and Ar treatments caused foam breakage, the time from foam start until the foam covered the pigs' withers was recorded. This time differed significantly ($P < 0.001$) between all three treatments, with LSM ± SE 42.6 ± 4.30 s for CO_2, 58.2 ± 4.37 s for N_2 and 74.5 ± 4.38 s for Ar. Foam filling time was included as a covariate in the models analysing both continuous duration (Model 1), and binomial behaviour (Model 2) response variables. Foam filling time had a significant ($P < 0.05$) effect on the occurrence of the behaviours explore foam and explore floor, and on the latencies from foam start to LOB and from LOB to LOP.

Before foam production began, ambient oxygen (O_2) concentrations in the box were above 20%. If many foam bubbles broke early during filling, the O_2 concentrations would start decreasing before the foam reached the air sampling inlet. When the foam reached the inlet, the O_2 concentration should drop immediately to zero. However, the sampling tube, water trap, and moisture filter were initially filled with air, causing a delay before measuring the actual gas concentration inside the container. On average, the delay from when O_2 concentration fell from 20% to below 2%, was 43 s. Despite the water trap and moisture filters, the wet and humid conditions in the box caused the sensor to malfunction after 3–4 tests each day, and it stopped working entirely by the fourth day, resulting in only 11 reliable measurements. After the box was filled and foam production stopped, and the gas pulse was used to break the foam, O_2 concentrations in the box were below 1% and remained at that level for the duration of the test until the box was opened to remove the pig.

At the start of each test, the average temperature in the box was 15.1 °C, slightly lower for the first test in the morning and increasing during the day. After the box was filled and the foam was destroyed, the temperature rose slightly to 15.4 °C. The cooling effect of the compressed gas and the water/soap mixture used to create the foam did not significantly impact the temperature in the container.

Pig behaviour

The behaviours ‘startle’, ‘shake’, retreat, ‘explore lid’, ‘defecate’ and ‘gasping’ occurred infrequently and were not analysed further. ‘Startle’ was observed in ten pigs in relation to the start of foam production. ‘Retreat’ may have been limited due to the confined space. Most pigs (72%) occasionally slipped, usually when foam levels were high. ‘Gasping’ was observed in one pig (CO_2 treatment), but the behaviour was perceived as difficult to detect due to the video angles.

Explore window and foam

The transparent window between containers was explored by most pigs across treatments. Interest in the window was highest during the first intervals and decreased as foam filled the container (Fig. 2). In the CO_2 treatment, the behaviour decreased more rapidly compared to N_2 and Ar, as can be seen from interval 6 and onward. Only one pig never explored the window and two pigs only explored it before foam production started (intervals 1–3).

The behaviour ‘explore foam’ was observed in all treatments, but six pigs never explored the foam, of which 5 were in the CO_2 and one in the N_2 treatment. In the CO_2 treatment, the exploration of foam had a sharp peak in interval 5 and then rapidly decreased, while the behaviour was shown more persistently in the N_2 and Ar treatment (Fig. 3).

Avoid foam

All pigs (100%), across treatments performed ‘avoid foam’ at least once during the observation period. The percentage of pigs showing the behaviour during each interval is shown in Fig. 4. In the CO_2 treatment, ‘avoid foam’ peaked earlier than in the N_2 and Ar treatments and in intervals 7 and 8, all pigs in the CO_2 treatment showed the behaviour. ‘Avoid foam’ peaked in intervals 8, 9 and 10 in N_2 and in interval 10 in the Ar treatment, i.e. when foam levels were quite high.

Escape attempts

Escape attempts were observed at least once in 42% of pigs in N_2, 58% of pigs in Ar and 83% of pigs in CO_2 treatment. Pigs in the CO_2 treatment showed escape attempts significantly earlier than the other two treatments and a majority of the pigs showed the behaviour in intervals 7 and 8, with a rapid decrease after that as a result of LOP (Fig. 5). Escape attempts peaked in interval 10 in the N_2 and interval 11 for the Ar treatment.

Vocalisation

There were no differences between treatments in the number of grunts or screams before LOP (Kruskal-Wallis Test, P = 0.993, N =

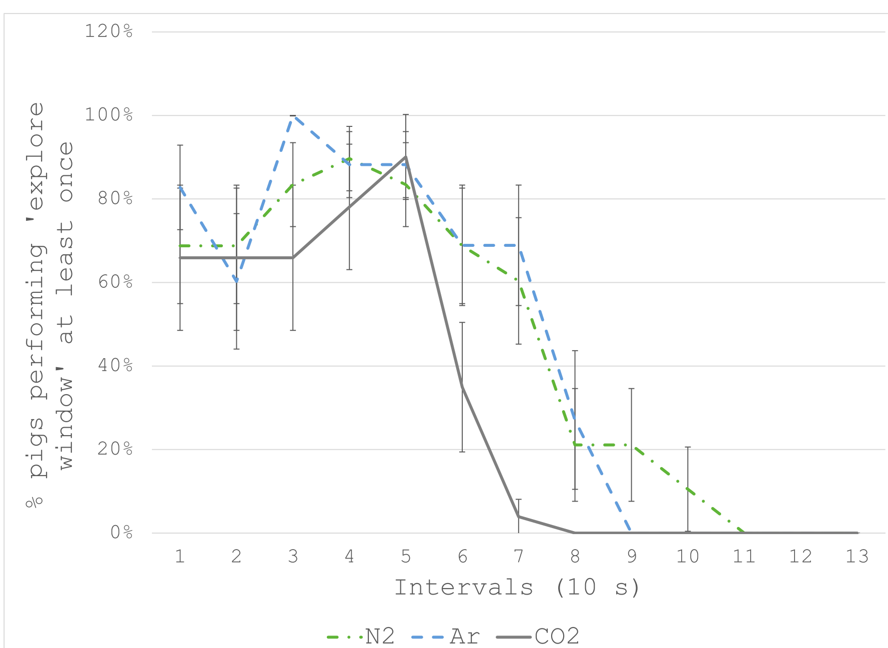

Fig. 2. Percentage of pigs (Least Square Means and SE) performing the behaviour ‘explore window’ at least once during each 10-second interval (1–13) when exposed to N_2, Ar or CO_2. Interval 4 indicates foam start. Significant difference (P < 0.05) is indicated when SE bars do not overlap, both between treatments within interval and between intervals within treatment.

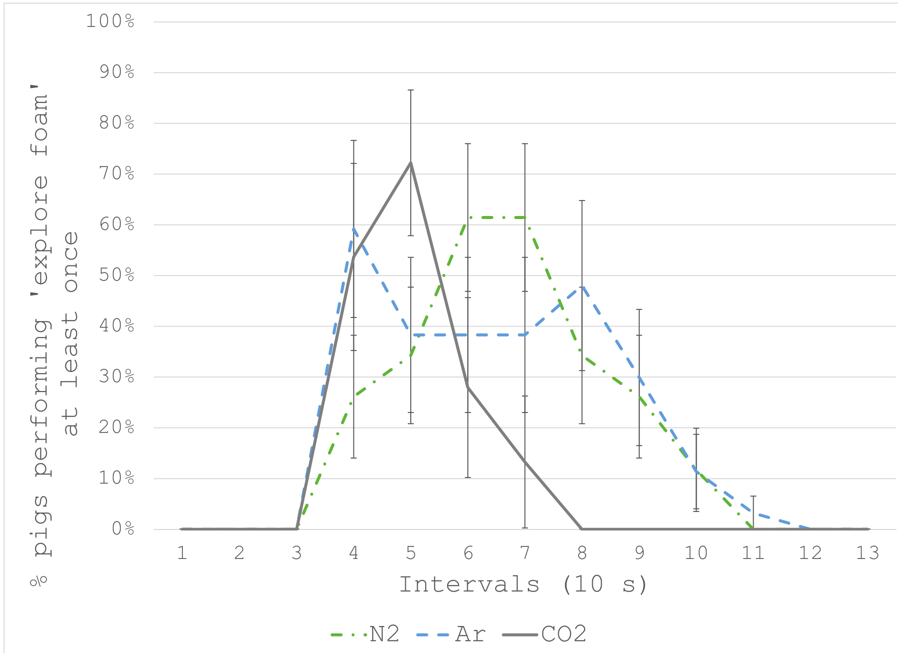

Fig. 3. Percentage of pigs (Least Square Means and SE) performing the behaviour 'explore foam' at least once during each 10-second interval (1–13) when exposed to N_2, Ar or CO_2. Interval 4 indicates foam start. Significant difference ($P < 0.05$) is indicated when SE bars do not overlap, both between treatments within interval and between intervals within treatment.

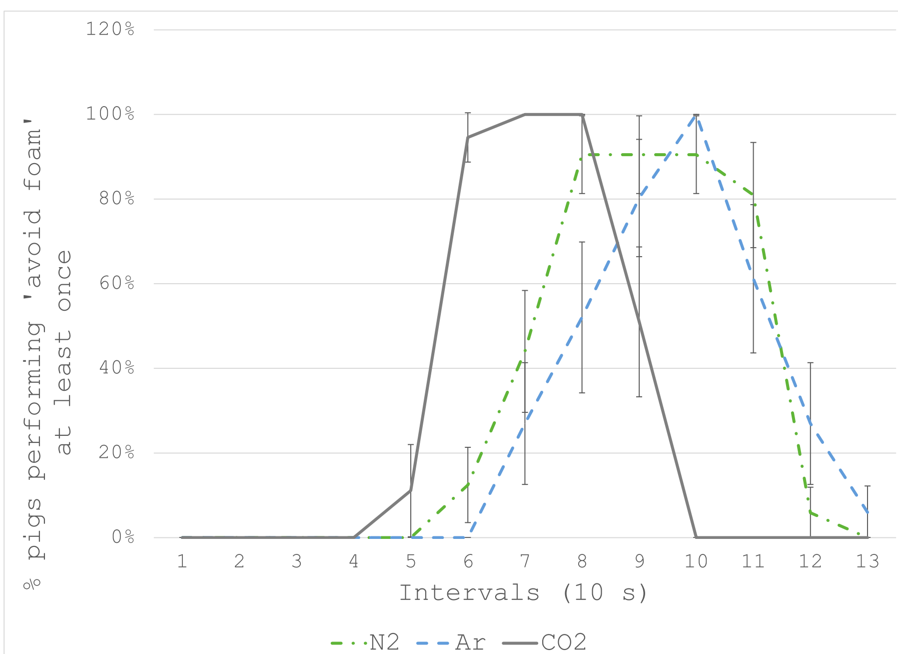

Fig. 4. Percentage of pigs (Least Square Means and SE) performing the behaviour 'avoid foam' at least once during each 10-second interval (1–13) when exposed to N_2, Ar or CO_2. Interval 4 indicates foam start. Significant difference ($P < 0.05$) is indicated when SE bars do not overlap, both between treatments within interval and between intervals within treatment.

36). After LOP, pigs in the N_2 and Ar treatments performed high-pitch vocalisations (screams) more frequently than pigs in the CO_2 treatment (Kruskal-Wallis Test, $P = 0.002$, CHI-square = 12.5, N = 36). The median values were 3.5, 3.0 and 0.0, and the interquartile range values were 4.5, 5.0 and 0.0 for the N_2, Ar and CO_2 treatments, respectively. Only two pigs (one in the Ar and one in the CO_2 treatment) grunted after LOP.

Loss of balance, loss of posture, and convulsions

The stun process was similar between pigs in the N_2 and Ar treatment, while the CO_2 treatment differed. The first sign of the anoxic atmosphere's effect was LOB, where pigs began to sway, stagger, sit and fall while struggling to keep an upright position. Some pigs showed potentially uncontrolled muscle contractions

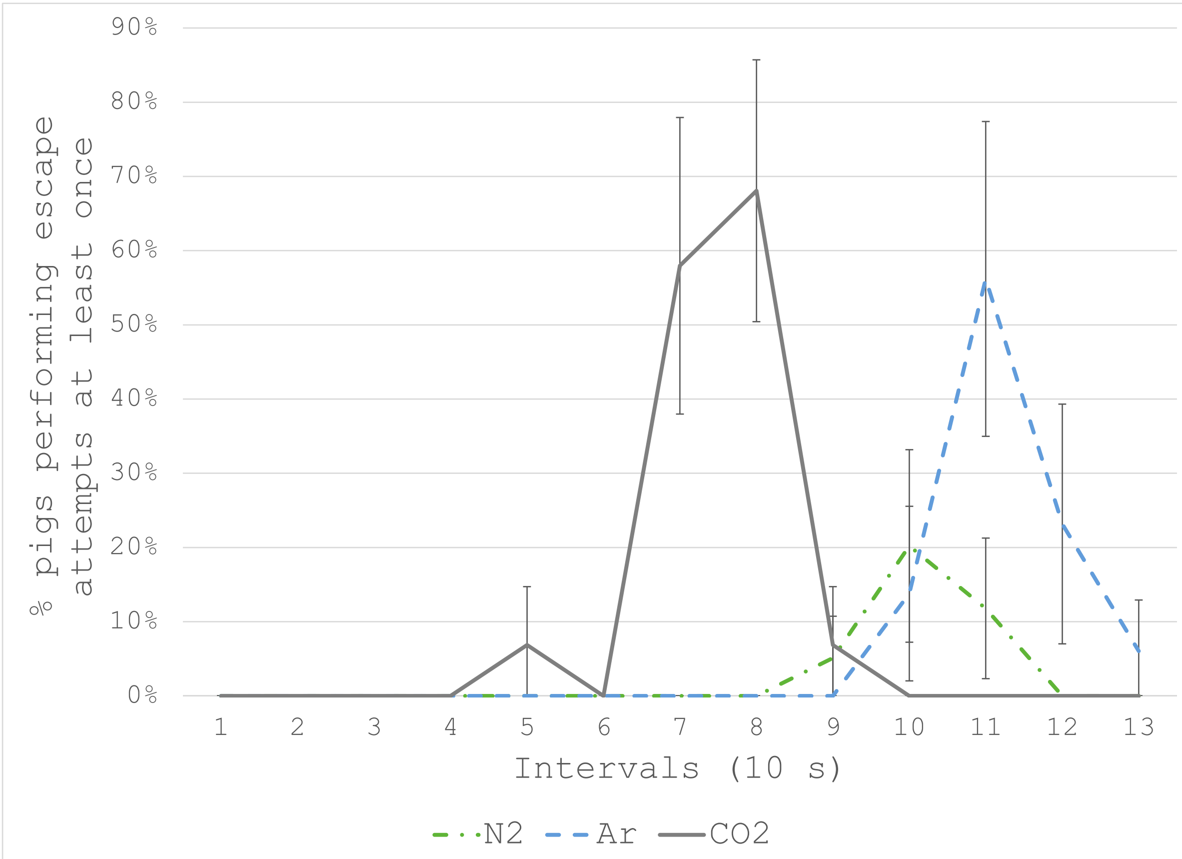

Fig. 5. Percentage of pigs (Least Square Means and SE) performing escape attempts at least once during each 10-second interval (1–13) when exposed to N_2, Ar or CO_2. Interval 4 indicates foam start. Significant difference (P < 0.05) is indicated when SE bars do not overlap, both between treatments within interval and between intervals within treatment.

Table 2
Latencies (s) from foam start to LOB, LOP, last strong convulsion and last muscle contraction of pigs in the three treatments N_2, Ar and CO_2. Latencies from LOB to LOP and from LOP to last strong convulsion and last muscle contraction are also shown.

	Treatment							
	N_2		Ar		CO_2			
Latency (s)	LSM	SE	LSM	SE	LSM	SE	F	P-value
From foam start to:								
LOB	62.5[a]	2.76	67.3[a]	3.25	31.7[b]	3.28	26.7	<0.001
LOP	77.2[a]	2.92	76.8[a]	3.44	51.1[b]	3.47	15.1	<0.001
Last strong convulsion	102.5[a]	2.90	94.1[a]	3.92	56.1[b]	5.78	23.7	<0.001
Last muscle contraction	208.3[a]	9.66	218.1[a]	11.38	167.3[b]	11.46	4.2	0.032
From LOB to:								
LOP	14.7[ab]	2.46	9.47[a]	2.90	19.4[b]	2.92	2.4	0.123
From LOP to:								
Last strong convulsion	23.4[a]	2.52	12.9[b]	3.40	12.7[ab]	5.02	6.5	0.010
Last muscle contraction	131.1	10.02	141.3	11.81	116.3	11.89	0.9	0.438

Abbreviations: LOB = Loss of balance; LOP = Loss of posture.
[a–b] Values within a row with different superscripts indicate pairwise differences with P < 0.05.

before LOP. In the N_2 and Ar treatment, LOP was followed by the immediate onset of strong convulsions, including paddling (galloping) movements with the legs, often with a high-pitched scream. These muscle contractions then became more irregular, followed by gagging. In the CO_2 treatment, pigs generally had fewer and less severe convulsions and no vigorous paddling movements.

The time from foam start to LOB, LOP, last strong convulsion and last visible muscle contraction was shorter for the CO_2 treatment compared to N_2 and Ar (P < 0.001; Table 2). No differences were found between the N_2 and Ar treatments (Table 2). Latency from LOB to LOP did not differ between treatments.

Table 2 also presents latencies of convulsions from LOP. The duration of strong convulsions was longer in the N_2 treatment compared to Ar (P < 0.001), while no difference was found compared to the CO_2 treatment although the mean duration was similar to the Ar treatment. There was no difference between treatments in duration until the last observed muscle contraction.

By the end of the exposure time, pigs were checked for corneal reflexes, muscle contractions and gagging/breathing, and heartbeats using a stethoscope. All pigs lacked corneal reflex, and none showed signs of breathing or muscle contractions.

Blood lactate analysis

The average blood lactate concentrations were 12.7 ± 1.23, 11.4 ± 1.35 and 9.1 ± 1.40 mM in the N_2, Ar and CO_2 treatment, respectively, without significant differences between treatments ($P = 0.239$). Regression analysis showed no association between lactate concentrations and time to LOB, LOP, last strong convulsion or last observed muscle contraction.

Discussion

Two behaviours considered to be signs of aversion in pigs during controlled atmosphere stunning are retreat and escape attempts (Raj and Gregory, 1996; Llonch et al., 2012b). The results showed that pigs in the CO_2 treatment performed escape attempts significantly earlier than in N_2 and Ar, and there were also more pigs that exhibited escape attempts in CO_2, supporting the conclusion that high concentration of CO_2 is more aversive to inhale than the inert gases and in particular N_2 gas (EFSA, 2004). However, 42% of pigs exposed to N_2 foam still demonstrated at least one escape attempt before LOP. Lindahl et al. (2020) found no difference in escape attempts between 9-week-old pigs exposed to air-filled and N_2-filled foam, concluding the aversion was related to the foam itself. As in Lindahl et al. (2020), the current study observed increased escape attempts as foam filled the container, with pigs across treatments avoiding the foam when it reached the head. Despite this, some pigs voluntarily immersed their head into the foam. Retreat was rarely observed, likely due to the confined space of the container. Thus, the results indicate that the pigs seem to experience being enveloped by the foam as unpleasant, but Ar and N_2 induce a less aversive response in comparison to CO_2.

Vocalisation is often considered a conscious response (Verhoeven et al., 2015) and can indicate distress during e.g., captive bolt stunning (Gouveia et al., 2009). However, high-pitched sounds after LOP during gas stunning are likely involuntary passage of air along the vocal cord (Verhoeven et al., 2015). For example, Raj (1999) studied pigs exposed to anoxia induced by Ar and reported that unconsciousness occurred before the onset of vocalisations. It is therefore relevant to distinguish between vocalisations before and after LOP in gas stunning, which was done in the present study. Grunts and screams before LOP occurred in all treatments with no significant differences, while high-pitched sounds after LOP only occurred in inert gas treatments. However, due to the uncertainty regarding the level of consciousness during high-pitched vocalisations in relation to LOP, this seems to be an inconclusive indicator, something that has also previously been acknowledged by e.g., Llonch et al. (2012b). In addition, although conscious vocalisations may be indicators of distress, various explanations to an absence of vocalisations have been proposed (Verhoeven et al., 2015), and the absence of vocalisations cannot be interpreted as proof of absence of distress. For example, pain from inhaling high CO_2 concentrations may prevent pigs from vocalising (Llonch et al., 2012b). Grunting may be a relevant indicator of consciousness and, in the present study, grunts mainly occurred before LOP and sounds similar to grunts after LOP were most likely the typical snoring sounds related to gagging (thus not conscious vocalisations).

Gasping, considered an indicator of breathlessness (Velarde et al., 2007; Verhoeven et al., 2015) and commonly reported in pigs inhaling CO_2 (Atkinson et al., 2020; Jongman et al., 2021; Velarde et al., 2007), was observed only in one pig exposed to CO_2 in this study. Terlouw et al. (2021) exposed pigs to different CO_2 gas mixtures and concluded that all mixtures induced respiratory difficulties in most pigs before loss of consciousness. Inert gases do not irritate mucosa and airway passages as CO_2 does, thus breathlessness and gasping are not induced to the same extent during inert gas hypoxia. Several studies have shown decreased gasping with decreased CO_2 concentration in the gas mixture (Llonch et al., 2012a, 2012b; Atkinson et al., 2020). The CO_2 treatment is an example of hypercapnic anoxia, as CO_2 concentrations are so high that an anoxic situation is created, thus, the level of residual oxygen was similar in all gases. Absence of gasping was also found in a previous study of N_2 gas anoxia using gas-filled foam (Lindahl et al., 2020).

Social isolation distress in pigs is well documented. Söderquist et al. (2023) found fewer escape attempts in pigs exposed to air-filled foam when accompanied by a familiar pig. In the present study, pigs had contact with a familiar pig through a clear perforated window, which may have provided social support. This is suggested by only 42% of pigs in the N_2 treatment showing escape attempts, compared to 80% in isolated pigs from Lindahl et al. (2020). In future tests, the two containers should be connected by the short side of the container, so the pigs are naturally facing each other.

In a controlled atmosphere stunning, the earliest behavioural sign of the onset of unconsciousness is LOP (Verhoeven et al., 2015; Raj, 1999; Raj and Gregory, 1996; EFSA, 2013). Studies have shown that the time to LOP in pigs is longer using inert gases compared to CO_2, due to the anaesthetic effect of CO_2 (Raj and Gregory, 1996; Llonch et al., 2012b). This was confirmed in this study where LOP occurred earlier in the CO_2 treatment with a time difference of about 26 s compared to Ar and N_2. It should be noted that the time to LOP is not directly comparable to non-foam studies, as it was measured from foam production start, introducing a delay until true anoxic conditions were achieved. In the present study, time to LOP was 51.2 s in the CO_2 treatment, to be compared with approximately 30 s when pigs are exposed to 80–90% CO_2 gas without foam (Mota-Rojas et al., 2012). The filling time of the container is a key factor for the time to LOP in the gas foam method (Lindahl et al., 2020). Accordingly, foam filling time had a significant effect on the latencies from foam start to LOB and from LOB to LOP in the current study. However, as the treatment effect with a shorter time to LOP for CO_2 compared to Ar and N_2 is still present with adjustment of filling time, filling time is not the only contributing factor to the shorter time to LOP for CO_2. Lindahl et al. (2020) found the time to LOP for 30 kg pigs exposed to N_2 gas in foam to be 58 s, which can be compared to 77 s in the present study with 50 kg pigs.

During gas stunning, pigs commonly show ataxia, poor muscle control, before LOP as the induction of unconsciousness is progressive. Time to LOB was shorter in CO_2 treatment than in Ar and N_2. During the period from LOB to LOP, the pigs showed behaviours like swaying, sitting, falling and getting up again, escape attempts and muscle excitation. This period of high activity makes it difficult to differentiate specific behaviours. There is a lack of agreement in the scientific literature on whether muscular excitation occurs as a voluntary response to the gas or if it is a period of involuntary movements, which is problematic as this is crucial for assessing the level of discomfort that the animals experience before the loss of consciousness. Forslid (1987) suggested, based on EEG, that pigs exposed to CO_2 were unconscious before the muscular excitation period and several studies have also implied LOP as an indicator of successful stunning that shows that the cerebral cortex is no longer able to control posture (Raj and Gregory, 1996; Llonch et al., 2013). Conversely, results from more recent studies of brain activity of pigs during exposure to different concentrations of CO_2

and CO_2 and N_2 mixtures, suggest that muscle excitation and LOP may occur before significant changes in brain function, which could indicate that the pigs are conscious (Rodríguez et al., 2008; Verhoeven et al.,2016). The dispute can partly be explained by difficulties determining the exact moment, based on the EEG data, at which loss of consciousness occurs (Rodríguez et al., 2008) and furthermore the difficulty of determining the exact time of LOP (EFSA, 2013). However, a recent study by Wabakken Hognestad et al. (2023) showed that some pigs stunned with CO_2 inhalation performed forceful galloping movement when blood pH and partial pressure of arterial CO_2 were considered compatible with consciousness.

In the current study, although ataxia was induced earlier in CO_2, the latency between the start of LOB to LOP did not differ between treatments. The muscle excitation phase from LOP to the last strong convulsion was longer for inert gases than CO_2, aligning with previous studies (Llonch et al., 2012b; 2013). The period of vigorous muscle contractions was commonly followed by gagging, i.e. low-frequency inhalations with opening/closing of mouth and occasional emission of sounds similar to snoring. Gagging may be similar to the behaviour 'agonal gasping' described in Wabakken Hognestad et al. (2023), which also occurred late in the stunning process. Agonal gasping was defined as "opening mouth wide and inhaling while having stopped breathing spontaneously in between mouth openings", which is in line with the observations of gagging in the current study. In Wabakken Hognestad et al. (2023), the onset of agonal gasping in pigs during CO_2 stunning was shown to correspond with low blood pH and high CO_2 partial pressure in the blood which were assessed as most likely incompatible with consciousness. There seems to be some conceptual confusion regarding gagging and gasping across studies, as different studies use different definitions, which highlights a need for consensus regarding these concepts in future. The results showed that all pigs across treatments were adequately stunned after 5 min from foam start and none of the pigs showed corneal reflex, breathing, gagging or any muscle contraction when pulled out of the container. Heartbeats were absent in a majority of the pigs; however, heartbeats could have been missed due to the difficulty of detecting weak or infrequent beats. No pig showed indications of regained consciousness during sticking and bleeding.

Blood lactate concentration, an indicator of stress shown to be related to specific handling and behaviour in a commercial slaughter setting (Brandt and Aaslyng, 2015; Edwards et al., 2010b) did not differ between treatments despite differences in duration of strong convulsions. Tonic-clonic seizures in humans are associated with strong sustained convulsions that cause significant lactate elevation in blood (Nass et al., 2019). This is very similar to the muscle excitation phase after LOP during stunning with inert gases. To the authors' knowledge, there are no studies of how convulsions during anoxic atmosphere stunning of pigs affect blood lactate concentrations, but it is likely that the lactate concentrations in this study were more influenced by physical factors than psychological stress, thus the inclusion of additional stress indicators is recommended for future studies. Various physiological variables in relation to stunning with CO_2 mixtures have been studied in e.g., Terlouw et al. (2021) and Wabakken Hognestad et al. (2023). Blood lactate concentration at exsanguination has been shown to be related to meat quality parameters (Edwards et al., 2010a; Hambrecht et al., 2004), and is thus a relevant factor when considering the N_2 foam method for stunning purposes at slaughter.

This study serves as a proof of concept of the NEFS in container method for stunning and subsequent killing of pigs of approximately 50 kg live weight. The system worked well in achieving and maintaining an anoxic atmosphere of below 2% O_2 throughout the procedure. The problems encountered with the oxygen sensor during the experiments may be eliminated by using a sensor designed for measurements in both liquids and gases. Continuously monitoring and maintaining O_2 levels below 2% in the foaming container throughout the process is one of the recommendations stated in the scientific opinion on NEFS (EFSA, 2024).

Conclusion

The gas foam method is effective in stunning the pigs regardless of the gas type used. All pigs across treatments were adequately stunned or dead when removed from the container 5 min after foam initiation, and none showed signs of regained consciousness during sticking and bleeding. The pigs exhibited behaviours indicative of a stronger aversion when exposed to CO_2 foam, especially compared to N_2. However, the stun process was faster with CO_2 where pigs displayed LOP, i.e. first indication of unconsciousness, after significantly shorter exposure time compared to the inert gases. The less aversive responses to Ar and N_2 foam are positive from an animal welfare perspective, but the longer time to loss of consciousness compared to CO_2 is a disadvantage.

Ethics approval

All procedures in this study complied with the Swedish and European Union regulations on animal experiments and were approved by the ethical committee in Uppsala, Sweden (ref. no.5.8.18-13402/2021; approval date 27 August 2021).

Data and model availability statement

None of the data were deposited in an official repository but are available from the corresponding author upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

CL: https://orcid.org/0000-0003-3748-3918.
ES: https://orcid.org/0000-0003-2313-7512.
MG: https://orcid.org/0000-0002-4410-0818.
CB: https://orcid.org/0000-0001-5671-5273.
AW: https://orcid.org/0000-0001-8012-2849.

CRediT authorship contribution statement

C. Lindahl: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualisation. **E. Sindhøj:** Writing – review & editing, Methodology, Investigation, Data curation. **M.A. Gerritzen:** Writing – review & editing, Methodology, Investigation. **H.G.M. Reimert:** Investigation, Data curation. **C. Berg:** Writing – review & editing, Methodology, Funding acquisition. **M. Blad:** Writing – review & editing, Investigation. **A. Wallenbeck:** Writing – review & editing, Visualisation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualisation.

Declaration of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Acknowledgements

We would like to thank Marianne Tersmeden, RISE, and Ulrika Nielsen, SLU, for help in conducting the practical experiments, and all the staff at the Swedish University of Agricultural Sciences' pig facility, SLU Lövsta, who supported us throughout the study. We would also like to thank Robin Naviga and Rasmus Nyberg Sörensen at HEFT AB for the technical support and production of custom-made container designs to fulfil the specific needs of the study.

Financial support statement

This work was supported by Formas (grant number 2020-02554).

References

Atkinson, S., Algers, B., Pallisera, J., Velarde, A., Llonch, P., 2020. Animal welfare and meat quality assessment in gas stunning during commercial slaughter of pigs using hypercapnic-hypoxia (20% CO_2 2% O_2) compared to acute hypercapnia (90% CO_2 in Air). Animals 10, 2440. https://doi.org/10.3390/ani10122440.

Brandt, P., Aaslyng, M.D., 2015. Welfare measurements of finishing pigs on the day of slaughter: a review. Meat Science 103, 13–23.

Dalmau, A., Llonch, P., Rodríguez, P., Ruíz-de-la-Torre, J.L., Manteca, X., Velarde, A., 2010a. Stunning pigs with different gas mixtures: gas stability. Animal Welfare 19, 315–323.

Dalmau, A., Rodriguez, P., Llonch, P., Velarde, A., 2010b. Stunning pigs with different gas mixtures: aversion in pigs. Animal Welfare 19, 325–333.

Edwards, L.N., Engle, T.E., Correa, J.A., Paradis, M.A., Grandin, T., Anderson, D.B., 2010a. The relationship between exsanguination blood lactate concentration and carcass quality in slaughter pigs. Meat Science 85, 435–440.

Edwards, L.N., Grandin, T., Engle, T.E., Ritter, M.J., Sosnicki, A.A., Carlson, B.A., Anderson, D.B., 2010b. The effects of pre-slaughter pig management from the farm to the processing plant on pork quality. Meat Science 86, 938–944.

EFSA, 2004. Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal 45, 1–29. https://doi.org/10.2903/j.efsa.2004.45.

EFSA, 2013. Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing. EFSA Journal 11, 3486.

EFSA, 2024. The use of high expansion foam for stunning and killing pigs and poultry. EFSA Journal 22, e8855. https://doi.org/10.2903/j.efsa.2024.8855.

Forslid, A., 1987. Transient neocortical, hipocampal and amygdaloid EEG silence induced by one minute inhalation of high concentration CO_2 in the swine. Acta Physiologica Scandinavica 130, 1–10.

Gouveia, K.G., Ferreira, P.G., Roque de Costa, J.C., Vaz-Pires, P., Martins da Costa, P., 2009. Assessment of the efficiency captive-bolt stunning in cattle and feasibility of associated behavioural signs. Animal Welfare 18, 171–175.

Hambrecht, E., Eissen, J.J., Nooijent, R.I.J., Ducro, B.J., Smits, C.H.M., den Hartog, L.A., Verstegen, M.W.A., 2004. Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. Journal of Animal Science 82, 1401–1409. https://doi.org/10.2527/2004.8251401x.

HEFT, 2022. C1. Retrieved on 15 November 2022 from: https://heftinternational.com/wp-content/uploads/2022/10/HEFT-C1_Web.pdf.

HEFT, 2023. Retrieved on 15 January 2024 from: https://heftinternational.com.

Jongman, E.C., Woodhouse, R., Rice, M., Rault, J.-L., 2021. Pre-slaughter factors linked to variation in responses to carbon dioxide gas stunning in pig abattoirs. Animal 15, 100134. https://doi.org/10.1016/j.animal.2020.100134.

Lindahl, C., Sindhøj, E., Brattlund Hellgren, R., Berg, C., Wallenbeck, A., 2020. Responses of pigs to stunning with nitrogen filled high-expansion foam. Animals 10, 2210. https://doi.org/10.3390/ani10122210.

Llonch, P., Rodríguez, P., Gispert, M., Dalmau, A., Manteca, X., Velarde, A., 2012a. Aversion to nitrogen and carbon dioxide mixtures for stunning pigs. Animal Welfare 21, 33–39.

Llonch, P., Rodríguez, P., Gispert, M., Dalmau, A., Manteca, X., Velarde, A., 2012b. Stunning pigs with nitrogen and carbon dioxide mixtures: effects on animal welfare and meat quality. Animal 6, 668–675.

Llonch, P., Rodriguez, P., Jospin, M., Dalmau, A., Manteca, X., Velarde, A., 2013. Assessment of unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures. Animal 7, 492–498.

McKeegan, D.E.F., Reimert, H.G.M., Hindle, V.A., Boulcott, P., Sparrey, J.M., Wathes, C. M., Demmers, T.G.M., Gerritzen, M.A., 2013. Physiological and behavioural responses of poultry exposed to gas-filled high expansion foam. Poultry Science 92, 1145–1154. https://doi.org/10.3382/ps.2012-02587.

Mota-Rojas, D., Bolaños-López, D., Concepción-Méndez, M., Ramírez-Telles, J., Roldan-Santiago, P., Flores-Peinado, S., Mora-Medina, P., 2012. Stunning swine with CO_2 gas: controversies related to animal welfare. International Journal of Pharmacology 8, 141–151.

Nass, R.D., Zur, B., Elger, C.E., Holdenrieder, S., Surges, R., 2019. Acute metabolic effects of tonic-clonic seizures. Epilepsia Open 4, 599–608. https://doi.org/10.1002/epi4.12364.

Raj, A.B.M., 1999. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. The Veterinary Record 144, 165–168.

Raj, A.B.M., Gregory, N.G., 1996. Welfare implications of gas stunning of pigs. 2. Stress of induction of anaesthesia. Animal Welfare 5, 71–78.

Rodríguez, P., Dalmau, A., Ruiz-de-la-Torre, J.L., Manteca, X., Jensen, E.W., Rodríguez, B., Litvan, H., Velarde, A., 2008. Assessment of unconsciousness during carbon dioxide stunning in pigs. Animal Welfare 17, 341–349.

SAS, 2021. SAS user's guide, version 9.4. Statistical Analysis Systems Institute Inc., Cary, NC, USA.

Sindhøj, E., Lindahl, C., Bark, L., 2021. Review: Potential alternatives to high-concentration carbon dioxide stunning of pigs at slaughter. Animal 15, 100164. https://doi.org/10.1016/j.animal.2020.100164.

Söderquist, A., Wallenbeck, A., Lindahl, C., 2023. Social support in a novel situation aimed for stunning and euthanasia of pigs may be increased by familiar pigs - a behavioural study with weaners. Animals 13, 481. https://doi.org/10.3390/ani13030481.

Steiner, A.R., Flammer, S.A., Beausoleil, N.J., Berg, C., Bettschart-Wolfensberger, R., Pinillos, R.G., Golledge, H.D.R., Marahrens, M., Meyer, R., Schnitzer, T., Toscano, M.J., Turner, P.V., Weary, D.M., Gent, T.C., 2019. Humanely ending the life of animals: research priorities to identify alternatives to carbon dioxide. Animals 9, 911.

Swedish University of Agricultural Sciences, 2017. Resources at the Swedish Livestock Research Centre. The Swedish Livestock Research Centre, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences. Retrieved on 27 November 2022 from Resources at SLU Lövsta march 2017 webb.pdf.

Terlouw, E.M.C., Deiss, V., Astruc, T., 2021. Stunning of pigs with different gas mixures: behavioural and physiological reactions. Meat Science 175, 108452. https://doi.org/10.1016/j.meatsci.2021.108452.

Velarde, A., Cruz, J., Gispert, M., Carrión, R., de la Torre, J.L., Diestre, A., Manteca, X., 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. Animal Welfare 16, 513–522.

Verhoeven, M.T.W., Gerritzen, M.A., Hellebrekers, L.J., Kemp, B., 2015. Indicators used in livestock to assess unconsciousness after stunning: a review. Animal 9, 320–330.

Verhoeven, M., Gerritzen, M., Velarde, A., Hellebekers, L., Kemp, B., 2016. Time to loss of consciousness and its relation to behavior in slaughter pigs during stunning with 80 or 95% carbon dioxide. Frontiers in Veterinary Science 3, 38. https://doi.org/10.3389/fvets.2016.00038.

Wabakken Hognestad, B., Digranes, N., Opsund, V.G., Espenes, A., Haga, H.A., 2023. CO_2 stunning in pigs: physiological deviations at onset of excitatory behaviour. Animals 13, 2387. https://doi.org/10.3390/ani13142387.

Wallgren, T., Wallenbeck, A., Berg, C., 2021. Stunning methods for pigs at slaughter. Report 56. Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Uppsala, Sweden.