Title: Identifying best operating procedures for CO_2 gas euthanasia of suckling and nursery age pigs – effects of stocking density - **NPB #12-100**

Investigators: Dr. Suzanne T. Millman, Dr. Larry Sadler

Institution: Iowa State University

Date Submitted: 04-29-2014

Industry Summary:
Gas euthanasia methods are commonly chosen when large groups of animals need to be humanely and efficiently put to death, such as during disease outbreaks. Nevertheless, previous research by our laboratory and others indicates that carbon dioxide gas does not provide instantaneous loss of consciousness and causes levels of distress in animals, including pigs. While the capacity to euthanize animals in groups is a major advantage of gas methods, group euthanasia demands consideration of both direct and bystander effects. The objective of this study was to evaluate the effects of chamber stocking rate on facets of animal welfare and efficacy during gas euthanasia of young pigs. Crossbred pigs (390 neonatal and 270 weaned) designated for euthanasia at production farms were randomly assigned to group sizes of one, two, four, or six pigs. Gas euthanasia of each piglet group was performed in a Euthanex® AgPro chamber. The chamber air was gradually displaced with CO_2 gas over 5 min to establish an in-chamber concentration of approximately 80% CO_2. Pigs remained in that atmosphere for an additional dwell period of at least 5 min. Higher stocking rates were associated with higher CO_2 concentrations after gradual fill for both age groups. While there was no evidence of an effect of stocking rate on latencies to loss of posture or last movement in suckling pigs, there was evidence of an effect on all measured efficacy variables in weaned pigs, with grouped pigs faster to succumb than solitary pigs. This finding is consistent with expected consequences of higher CO_2 concentration at increased stocking densities. Aversive states and behaviours of focal pigs in the chamber were scored from video. Weaned solitary pigs displayed a high incidence of pacing and may have experienced isolation distress. Escape attempts were absent in neonates and were not linearly affected by stocking rate in weaned pigs. Although the risk of hazardous interactions was correlated with group size, this study provided no evidence that isolation during gas euthanasia would benefit animal welfare.

The objective of the second phase of the study was to evaluate the effects of chamber stocking rate on facets of animal welfare and efficacy during euthanasia of weaned pigs with argon gas. Two hundred and thirty-three weaned pigs designated for euthanasia at a commercial production farm were randomly assigned to group sizes of one, two, or six pigs. Gas euthanasia of each piglet group was performed in a Euthanex® AgPro chamber. The chamber was pre-filled with argon gas for 6 min in order to reduce the oxygen concentration to less than 2%. Pigs were then placed into the pre-filled chamber and gas flow was continued at a high rate to displace any introduced air and re-establish a fatally low residual oxygen concentration. Pigs remained in the chamber for 10 min and then were removed to test for signs of sensibility and life. There was no significant evidence of an effect of stocking rate on focal pig latencies to onset of neuromuscular excitation or last movement, as scored from video recordings. Solitary pigs were more likely to pace and make righting attempts in the chamber than paired or grouped focal pigs, although pigs in higher stocking rate treatments tended to retain posture longer. The results of this study do not support seclusion during argon gas euthanasia as a method of improving animal welfare.

In conclusion, gas euthanasia of solitary pigs was associated with greater distress when using carbon dioxide or argon gases. Stocking density of grouped pigs did not affect distress associated with gas euthanasia when groups comprised 6 pigs or less.

Keywords:
euthanasia, pigs, stocking rate, carbon dioxide, argon

Scientific Abstract:

The objective of this study was to evaluate the effects of chamber stocking rate on facets of animal welfare and efficacy during gas euthanasia of young pigs. Crossbred pigs (390 neonatal and 270 weaned in Experiment 1 and 233 weaned pigs in Experiment 2) designated for euthanasia at production farms were randomly assigned to group sizes of one, two, four, or six pigs. Gas euthanasia of each piglet group was performed in a Euthanex® AgPro chamber. For Experiment 1b the chamber air was gradually displaced with CO_2 gas over 5 min to establish an in-chamber concentration of approximately 80% CO_2. Pigs remained in that atmosphere for an additional dwell period of at least 5 min. Higher stocking rates were associated with higher CO_2 concentrations after gradual fill for both age groups. While there was no evidence of an effect of stocking rate on latencies to loss of posture or last movement in neonatal pigs, there was evidence of an effect on all measured efficacy variables in weaned pigs, with grouped pigs faster to succumb than solitary pigs. This finding is consistent with expected consequences of higher CO_2 concentration at increased stocking densities. Aversive states and behaviours of focal pigs in the chamber were scored from video. Weaned solitary pigs displayed a high incidence of pacing and may have experienced isolation distress. Escape attempts were absent in neonates and were not linearly affected by stocking rate in weaned pigs. Although the risk of hazardous interactions was correlated with group size, this study provided no evidence that isolation during gas euthanasia would benefit animal welfare. For Experiment 2 the chamber was pre-filled with argon gas for 6 min in order to reduce the oxygen concentration to less than 2%. Pigs were then placed into the pre-filled chamber and gas flow was continued at a high rate to displace any introduced air and re-establish a fatally low residual oxygen concentration. Pigs remained in the chamber for 10 min and then were removed to test for signs of sensibility and life. There was no significant evidence of an effect of stocking rate on focal pig latencies to onset of neuromuscular excitation or last movement, as scored from video recordings. Solitary pigs were more likely to pace and make righting attempts in the chamber than paired or grouped focal pigs, although pigs in higher stocking rate treatments tended to retain posture longer. The results of this study do not support seclusion during argon gas euthanasia as a method of improving animal welfare.

Introduction:

Millions of pigs are euthanized every year by the U.S. swine industry. Blunt force trauma is currently the most common method used to euthanize compromised piglets, but this method can be aesthetically unpleasant and emotionally challenging to the individual performing the procedure (AVMA, 2007). The use of gas is becoming increasingly utilized by the U.S. swine industry, with carbon dioxide (CO_2) being the most commonly used gas. CO_2 has been shown to be a rapid depressant of the central nervous system with established analgesic and anesthetic properties. High concentrations of CO_2 cause depression of the central nervous system leading to loss of consciousness and subsequent death (Martloft 2002).

The humaness of CO_2 has been questioned since CO_2 is acidic and, as such, may irritate the mucus membranes. Some level of aversion or distress has been observed for all currently tested ages of pigs (Raj and Gregory, 1996; Sadler et al. 2011a; Sutherland. 2011). The use of argon has been proposed as an alternative gas for euthanizing compromised pigs. Argon is an odorless gas that induces insensibility and death through the displacement of oxygen (O_2). Sufficient research investigating the humanness and efficacy of argon is lacking. Weighing the relative humanness of different gases for induction of insensibility for anesthesia or euthanasia purposes has been a challenge for interpretation due to differences between types of behavior, physiologic and endocrine measures. Prior to encouraging U.S. farmers to change from CO_2 to argon euthanasia technologies, we must be confident that there is strong scientific evidence to support the perceived benefits to animal welfare.

Much of the current research on gas euthanasia has been conducted at processing facilities on market hogs, with CO_2 being studied for its effectiveness as a gas stunning agent and its effects on meat quality. Our research team has found that suckling and weaned piglets respond differently to the gas euthanasia process. For example, suckling pigs take less time to cease movement (which is usually respiration) and decrease the duration of open mouth breathing. Casual observation in the field suggest variability in responses of cull piglets to CO_2 euthanasia, with some producers reporting concerns about piglets surviving 15 minutes of exposure to CO_2 gas and requiring a second euthanasia procedure. This discrepancy highlights the importance to not extrapolate data for different ages of pigs and the need for refined standard operating procedures, engineering standards and for swine staff performing gas euthanasia on farm.

These occurrences pose problems for animal welfare and could arise due to physiologic and/or behavioral factors associated with the equipment or standard operating procedures. Guidance regarding the feasibility and welfare implications when euthanizing piglets in groups or singly has not been examined. This information is needed for the current industry standard, using CO_2 gas, as well as for evaluating novel gases such as argon, which may have prolonged induction periods.

Objectives:
There is a growing body of research for gas stunning and euthanasia of swine. However, empirical data has been primarily collected using controlled experiments in the laboratory or in meat processing facilities. The proposed research project addresses ethical and practical questions frequently posed to our research team by producers and veterinarians who are using or are considering converting to gas euthanasia for on-farm euthanasia of swine: How many pigs should be placed in the euthanasia box at the same time?

Materials & Methods:
This study is divided into two experiments: Experiment 1 (Exp 1) is testing CO_2 gas; Experiment 2 (Exp 2) is testing argon. Exp 1 is further broken down into two parts: Exp 1a is testing CO_2 in neonatal pigs (<72 hours); Exp 1b is testing CO_2 weaned pigs (3-10 weeks). Thirty replicates in each age group were tested.

Pigs were enrolled in order of health instability and euthanized in the same sequence. The treatment order applied to consecutively enrolled pigs was randomly determined. Treatments consisted of group sizes during euthanasia: one pig (TRT 1), two pigs (TRT 2), four pigs (TRT 4), or six pigs (TRT 6). Based on lack of significant findings from Exp 1a, TRT 4 was not used in Exp 1b or Exp 2. The treatment groups created variable chamber stocking rates. One randomly selected pig in each group was designated as the "focal pig" for collection of behavioural observations.

Experiment 1a

Crossbred ((Duroc) x (Large White x Landrace)) pigs of mixed sexes were utilized for this study only after being designated for euthanasia at commercial swine production farms due to animal welfare concerns or low commercial viability. A total of 390 pigs under 72 h of age (body weight mean with standard deviation: 0.79 ± 0.28 kg; range: 0.1 to 2.2 kg) were euthanized in Experiment 1. Most of the neonates used in this study could be characterized as low birth weight, low viability, or physically deformed at birth. Prior to selection, pigs were birthed and housed in indoor farrowing crates following standard husbandry practices of the producers. Upon designation for euthanasia at one of six proximal farrowing facilities, neonatal pigs were transported to a neighbouring building and group-housed with wood shavings and heat lamps during study enrolment.

Pigs were enrolled in the study shortly before euthanasia by identification and physical examination. Those animals with unstable vital signs or potentially painful injuries were granted antecedence for enrolment and pigs that appeared unable to withstand the enrolment process were rejected from the study and euthanized immediately in accordance with farm procedures. The enrolment examination included assessing and recording weight, sex, heart and respiratory rate, rectal body temperature, apparent reasons for selection, body condition, activity level, hydration status, and oral, ocular, and nasal discharges. Each enrolled pig was marked with a PaintStik® (ALL-WEATHER®, Elk Grove, USA), to correspond to its consecutive identification number. Table 1 includes severity scores used for clinical signs and codes used to designate reasons for selection. Table 2 provides the body condition scoring guidelines followed.

Gas euthanasia was performed in a 43 x 60 x 30 cm Euthanex® AgPro chamber with Smartbox™ control system (Value-Added Science & Technology, Mason City, USA) modified with transparent acrylic on the top and front panels for observation. Industrial grade CO_2 was supplied from compressed gas cylinders via a volumetric high-output heated two-stage regulator (Euthanex, Allentown, USA). A clean rubber mat and wood shavings were provided on the floor of the chamber prior to each trial to improve traction and comfort. The chamber was fitted with a carbon dioxide sensor

(CO2Meter, Ormond Beach, USA) attached to a HOBO data logger (Onset, Cape Cod, USA). Video recording was achieved with a portable observation lab (Noldus Information Technology, Wageningen, NL). Two colour digital video cameras (model WV-CP484, Panasonic, Kadoma, Japan) were positioned perpendicularly to view activity in the chamber from above and beside it. The cameras were connected to a multiplexer that enabled capture of a dual recording at 30 frames per second on a personal computer using HandiAvi software (v. 4.3, Anderson's AZcendant Software, Tempe, USA). Between each PG, the chamber was cleaned of bodily excretions and vacuumed to restore an ambient air composition.

During each trial, all of the pigs in a PG were enclosed in the chamber and the automated control box was used to time the administration of gas for 5 min. A flow duration of 5 min was established in pre-trial testing as the necessary time to reach an in-chamber concentration of 80% CO_2 at the flow rate of 1.59 m^3/h, or 35% chamber volume exchange per min, when the chamber was empty. A medium exchange rate of 35% was selected based on the results of prior research evaluating efficacy at different gas flow rates (see Sadler *et al* 2011a).

Based on National Pork Board recommendations to producers (2008), a 5 min in-chamber dwell period after reaching 80% CO_2 was considered adequate to achieve death. The first two neonatal piglet groups in Experiment 1 were left in the closed chamber for an additional 5 min in-chamber dwell; however, three of these six pigs (50%) had a detectable heartbeat upon removal. In an effort to achieve a lower failure rate, the dwell procedure was modified over the course of Experiment 1. The dwell period was first extended to 7 min for 10 PG and seven of these 25 pigs (28%) had a detectable heartbeat upon removal. The dwell period was further extended to 9 min for the next 23 PG, and 18 of these 80 pigs (23%) had a detectable heartbeat upon removal.

Since all piglets euthanized to that point had been insensible upon removal and all but one piglet had reached permanent respiratory arrest and presumably brain death by removal, it was decided that cardiac activity detectable by stethoscope was not indicative of unsuccessful gas euthanasia. All remaining piglets in Experiment 1 (85 PG, n = 279) were subject to 5 min of medium rate gas flow followed by a 5 min in-chamber dwell period during which a minimal gas flow rate (0.42 m^3/h, or 10% chamber volume exchange per min) was used to prevent a decline of the in-box CO_2 concentration below 80%.

Upon removal from the chamber, all pigs were immediately tested for signs of sensibility, including corneal reflex, withdrawal reflex to nose prick and leg pinch, and pupillary light reflex (National Pork Board 2008). Pigs were also observed for cessation of breathing and auscultated with a stethoscope for at least 30 s to confirm cardiac arrest. Pigs that had not permanently ceased respiration before removal from the chamber were immediately euthanized using methods approved in the AVMA Guidelines, namely additional gas exposure or blunt force trauma for neonates (2007). Pigs with a heartbeat but no detectable respiration upon removal were observed for a 10 min out-of-chamber dwell period in ambient air and then re-auscultated to confirm cardiac arrest. Pigs for which cardiac activity had not ceased after 10 min out-of-chamber were also secondarily euthanized with alternate, approved methods. No pigs recovered reflexes or resumed respiration during the out-of-chamber dwell period.

Experiment 1b

Methods used in Experiment 1b were the same as those used in Experiment 1a except as specified here. Experiment 1a used 270 post-weaning age pigs between 3-10 weeks of age (body weight mean with standard deviation: 4.0 ± 3.0 kg; range: 1.6 to 26 kg). Most of the weaned pigs used in this study were either stunted in growth, showing signs of respiratory disease, or suffering from an inguinal or umbilical hernia. Weaned pigs were enrolled and euthanized on a single farm while housed in indoor group nursery pens following standard husbandry practices of their producer.

Experiment 2 utilized a 61 x 91 x 46 cm chamber. In order to closely parallel Experiment 1, the flow rate needed to reach an in-chamber CO_2 concentration of 80% in 5 min was used. This was determined in pre-trial testing to be 4.53 m^3/h, or 30% chamber volume exchange per min, when the chamber was empty. Because post-weaning age pigs have been observed to lose posture and achieve last movement later than neonates (Sadler *et al* 2011b), all weaned pigs in Experiment 2 were left in the closed chamber for the 5 min gradual fill time plus an 8 min in-chamber dwell period prior to removal for sensibility testing.

Pigs that had not permanently ceased respiration by removal from the chamber were immediately euthanized using a penetrating captive bolt device per AVMA guidelines (2007). Pigs with a heartbeat but no detectable respiration upon removal were observed for a 10 min out-of-chamber dwell period in ambient air and then re-auscultated to confirm cardiac arrest. No weaned pigs were found to have cardiac activity lasting greater than 10 min after removal from the chamber and no pigs recovered reflexes or resumed respiration during the out-of-chamber dwell period.

Experiment 2

Methods used in Experiment 2 were the same as those used in Experiment 1b, except as specified here. Crossbred pigs of mixed sexes were utilized for this study after being designated for euthanasia at a commercial swine production farm due to animal welfare concerns or low commercial viability. Our request for locally-available weaned pigs slated for euthanasia but medically stable for transport obtained 233 animals for the study. The pigs were between 3-10 week of age (body weight mean with standard deviation: 7.7 ± 3.3 kg; range: 3.0 to 16.6 kg) and most showed symptoms of chronic respiratory, neurological, or dermatological disease. Cohorts of selected pigs were transported by livestock trailer and group-housed in an indoor biocontainment facility with ad libitum food and water during study enrolment.

Gas euthanasia was performed in a 61 x 91 x 46 cm Euthanex® AgPro chamber (Value-Added Science & Technology, Mason City, USA) modified with transparent acrylic on the top and front panels for observation. Argon gas is approved by the AVMA (2013) for pigs only if pigs are placed directly into an atmosphere of less than 2% residual oxygen, which necessitates pre-filling the chamber. A layer of transparent 20 g vinyl was fitted across the top of the chamber and attached with Velcro® tape to provide a barrier against air flow when the acrylic lid was opened after prefilling. An aperture of approximately 0.09 m^2 was cut through the vinyl layer in order to introduce animals. Industrial grade argon was supplied from compressed gas cylinders via a volumetric high-output heated two-stage regulator. A clean rubber mat and wood shavings were provided on the floor of the chamber prior to each trial to improve traction and comfort. The chamber was fitted with an oxygen sensor (CO2Meter, Ormond Beach, USA) attached to a HOBO data logger (Onset, Cape Cod, USA) that was set to record oxygen concentrations at 10 second intervals continuously on the days of the experiment. Between each PG, the chamber was cleaned of bodily excretions and vacuumed to restore an ambient air composition. A light was directed toward the front of the chamber with the room otherwise darkened to minimize visual disturbance of pigs in the chamber.

During each trial, argon gas was used to pre-fill the empty chamber at a flow rate of 0.12 m^3/min, or 49% chamber volume exchange per min, for 6 min. This established a residual O_2 concentration between 1-2%. After 6 min, the chamber lid was opened for approximately 20 s during which time either 6 pigs, 4 plastic barrels (7.6 L each) and 2 pigs, or 5 plastic barrels and 1 pig were placed inside. Plastic barrels were used to simulate the placement of additional animals in lower stocking rate treatments with the aim of minimizing differences in oxygen concentrations between treatments. When used, the barrels were always introduced first and were positioned along the back panel of the chamber. The barrels were weighted with sand to prevent movement by the pigs. The focal pig was placed last followed by immediate closure of the chamber lid. Gas flow was continued for approximately 2.5 min to displace introduced oxygen and re-establish an O_2 concentration between 1-2%. Pigs remained in the chamber for a total of 10 min.

Two PG trials were terminated due to interruptions of gas flow after animal placement. In one of these trials, the solitary pig was removed and euthanized immediately using a penetrating captive bolt device per AVMA Guidelines for Euthanasia (2013). In the other trial, gas flow was restored and the paired pigs were allowed to remain in the chamber until death had occurred. Behavioural data from these two trials were not used in analyses. All other pigs (n = 230) were insensible without respiration upon removal. One pig had a detectable heartbeat upon removal and was observed in ambient air and then re-auscultated 3 min later, by which time cardiac arrest had occurred.

Behavioural Observation

During exposure to the gases, a single unblinded observer recorded focal pig latency to loss of posture and latency to last movement. Latency to loss of posture was defined as the elapsed time from start of gas flow until the recumbent focal pig stopped attempting to lift its head. This behaviour was regarded as a non-invasive approximation of the time required to reach unconsciousness. Latency to last movement was defined as the elapsed time from start of gas flow to complete and permanent cessation of focal pig movement including respiratory arrest. This behaviour was regarded as a non-invasive approximation of the time required to achieve brain death.

Video recordings were scored by the same individual using Observer XT (Noldus, Wageningen, NL) for the frequency or duration of focal pig behaviours and statuses tied to specific animal welfare considerations. See Table 3 for video ethogram. An intra-observer reliability test performed in Observer generated a Cohen's *kappa* of 1.00 with a tolerance window of 3 s.

Statistical Analysis

Experiment 1

All statistical analyses were performed in SAS using a significance level of 0.05. The CO_2 concentrations recorded at conclusion of gradual fill (5 min from gas start) for each piglet group were analysed for differences in treatment means using a general linear model (PROC GLM) with stocking rate as a categorical variable and the sole fixed effect.

In behavioural data analyses, stocking rate was used as a continuous explanatory variable. Data were analysed for significant differences in focal pig responses. All clinical variables assessed during enrolment were examined as potential covariates. The final models were selected based on best fit and included weight, body condition score, and starting heart rate where specified in the results. All three latency variables were log-transformed to improve model fit.

Survival analysis with Weibull distribution (PROC LIFEREG) was used to test whether there was an association between CO_2 concentration at conclusion of gradual fill and latencies to loss of posture, onset of gasping (scored from video), and last movement (right censored at time of removal). Survival analysis (PROC LIFEREG) was also used to determine whether there was an effect of stocking rate on latencies to loss of posture, onset of gasping, or last movement. Levene's test for homogeneity of variance was used to test whether grouped focal pigs were more variable in latencies to loss of posture than solitary pigs (PROC GLM). Differences in failure rates (binary for heartbeat upon removal) by treatment were also analysed using survival analysis (PROC LIFEREG). Data analysis for failure rate during Experiment 1 used only the subset of 85 neonatal focal pigs that experienced a 5 min in-chamber dwell period with additional slow-flow CO_2.

Poisson regression (PROC GENMOD) was used to test for an effect of stocking rate on righting attempt and escape attempt counts, as scored from video. General linear modelling (PROC GLM) was used to test for an effect of stocking rate on the durations of open-mouth breathing, neuromuscular excitation, and pacing.

Experiment 2

All statistical analyses were performed in SAS® (SAS Inst. Inc., Cary, USA) using a significance level of 0.05. Oxygen concentrations recorded at 10 s intervals during the 10 min gas exposure were averaged to obtain one O_2 exposure value per PG trial. These O_2 exposure values were analysed for differences in treatment means using a general linear model (PROC GLM) with stocking rate and day of trial as categorical variables and fixed effects.

In behavioural data analyses, stocking rate was used as a continuous explanatory variable. Data were analysed for linear effect of stocking rate on focal pig responses. Clinical variables assessed during enrolment were examined as potential covariates along with day of trial. The final models were selected based on best fit and included day of trial and starting body temperature. Survival analysis with Weibull distribution (PROC LIFEREG) was used to determine whether there was an effect of stocking rate on latencies to onset of neuromuscular excitation, loss of posture, or last movement. All three latency variables were log-transformed to improve model fit. Levene's test for homogeneity of variance was used to test whether grouped and paired focal pigs were more variable in latencies to onset of neuromuscular excitation than solitary pigs (PROC GLM). Poisson regression (PROC GENMOD) was used to test for an effect of stocking rate on righting attempt counts. General linear modelling (PROC GLM) was used to test for an effect of stocking rate on the duration of neuromuscular excitation.

Results:

Experiment 1

Higher stocking rates in neonate trials were associated with higher CO_2 concentrations at conclusion of gradual fill (P = 0.003). The differences in gas concentration observed most likely reflect the change in stocking density created by increasing stocking rate; see Table 4 and Figure 1a for depictions of mean total body weight and CO_2 concentration by treatment. There was no significant evidence of an association between CO_2 concentration at conclusion of gradual fill and the log of any behaviour latencies in neonatal pigs after controlling for body condition score, weight, and starting heart rate (loss of posture P = 0.53; onset of gasping P = 0.47; last movement P = 0.99).

There also was no significant evidence of an effect of stocking rate on any behaviour latencies in neonates after controlling for body condition score, weight, and starting heart rate (loss of posture P = 0.06; onset of gasping P = 0.13; last movement P = 0.61). See Table 5 for mean latencies by treatment. There was no significant evidence of an effect of stocking rate on the proportion of neonatal pigs with heartbeat upon removal (P = 0.48).

The latencies to loss of posture for the first and last pig to succumb in each neonatal piglet group were recorded during live observation, and the difference between these values was calculated as an estimate of response asynchrony. Overall, the mean with standard error for the difference in responses within non-solitary neonatal piglet groups was 28 ± 2 s (range 2 to 66 s), with ⅓ of PG in TRT 6 having a difference between first and last latency to loss of posture that was greater than 45 s. There was no significant evidence of an effect of stocking rate on variability in latencies to loss of posture for neonatal pigs (P = 0.74).

Neonatal pigs were generally quiescent in the chamber and displayed few behaviours from which welfare could be assessed. Mean righting attempt counts were higher in solitary focal pigs than grouped focal pigs (P = 0.02). There was no significant evidence of an effect of stocking rate on durations of open-mouth breathing in neonates (P = 0.50). Escape attempts and pacing were not observed in this age group. Visible neuromuscular excitation was observed in only two of 120 focal pigs. Ataxia was not scored in this age group due to the prevalence of paresis and other causes of abnormal starting motor coordination in cull neonates. See Table 6 for a summary of behavioural data from neonatal focal pigs.

It was noted that the proportion of neonatal pigs receiving some type of potentially offensive contact from another pig while in the chamber ("strikes," eg, kicked, stepped on, or shoved) increased from 3% of focal pigs in TRT 2 to 30% of focal pigs in TRT 6. The average number of strikes received by each neonatal focal pig remained fairly low, but increased from 0.14 in TRT 2 to 0.60 in TRT 6.

Experiment 1b

As in neonate trials, higher stocking rates in post-weaning age trials were associated with higher CO_2 concentrations at conclusion of gradual fill (P < 0.0001); see Table 4 and Figure 1b for CO_2 concentration by total body weight of piglet groups. There was no significant evidence of an association between CO_2 concentration at conclusion of gradual fill and log latencies to loss of posture in weaned pigs after controlling for body condition score, weight, and starting heart rate (P = 0.30), but there was evidence of an association between the CO_2 concentration and log latencies to onset of gasping and last movement after controlling for body condition score, weight, and starting heart rate (onset of gasping P = 0.0009; last movement P < 0.0001).

There was evidence of an effect of stocking rate on the log of all behaviour latencies in weaned pigs after controlling for body condition score, weight, and starting heart rate (loss of posture P = 0.008; last movement P < 0.0001; onset of gasping P = 0.0004), where grouped focal pigs succumbed more quickly than solitary focal pigs. See Table 4 for mean latencies by treatment. There was also evidence of an effect of stocking rate on the proportion of weaned pigs with heartbeat upon removal using survival analysis (P = 0.04), with grouped focal pigs more likely to achieve cardiac arrest before removal. See Table 6 for proportions of pigs removed with heartbeat.

As in Experiment 1, differences between first and last latencies to loss of posture within PG were calculated to estimate response asynchrony. Weaned pigs had lower within-group variability than neonates. Across all non-solitary treatments, the mean with standard error for the difference in responses within weaned piglet groups was 17 ± 2 s (range 1 to 53 s), with only one PG in any treatment having a difference between first and last latency to loss of posture that was greater than 45 s. There was no significant evidence of an effect of stocking rate on variability in latencies to loss of posture for weaned pigs ($P = 0.08$), although the trend was toward decreased variability in TRT 6.

In Experiment 2, mean righting attempt counts were higher in grouped focal pigs than solitary focal pigs ($P = 0.04$). There was no significant evidence of an effect of stocking rate on escape attempt counts ($P = 0.72$). Mean duration of pacing was higher at lower stocking rates ($P < 0.001$), as was mean duration of neuromuscular excitation ($P = 0.02$). There was no significant evidence of an effect of stocking rate on durations of open-mouth breathing ($P = 0.08$) or ataxia ($P = 0.30$). See Table 7 for a summary of behavioural data from weaned focal pigs.

In CO_2 weaned pigs were both more active and more crowded in the chamber, and potentially negative interaction was much more common in weaned pigs than in neonatal pigs. The proportion of weaned pigs receiving some type of potentially offensive contact from another pig while in the chamber ("strikes," eg, kicked, stepped on, or shoved) increased from 34% of focal pigs in TRT 2 to 81% of focal pigs in TRT 6. The average number of potentially offensive contacts increased from 1.1 per pig in TRT 2 to 4.0 per pig in TRT 6. Many kicks were the result of neuromuscular excitation, which was observed before, during, and after loss of posture during CO_2 euthanasia, so it is not known how many of these strikes were consciously perceived

Experiment 2

O_2 data was successfully recorded during 44 of 76 PG trials. Within this subset, increased stocking rate was associated with higher average O_2 concentrations after controlling for day of trial ($P < 0.0001$), presumably due to the added time and chamber manipulation required to place multiple animals. Least square means for average O_2 exposure were 2.23 ± 0.04%, 2.28 ± 0.03%, and 2.44 ± 0.03% for TRT 1, TRT 2, and TRT 6, respectively. The estimated difference between TRT 6 and TRT 1 was 0.21 ± 0.05%. See Figure 2 for a representation of the pattern of O_2 concentration change during prefill, pig placement, and in-chamber dwell.

Escape attempts were not observed from any focal pigs during argon exposure. Brief periods of open-mouth breathing were scored in 7 of 74 focal pigs (9%). Typically, weaned pigs stood quietly until the onset of neuromuscular excitation. Twenty-one focal pigs (28%) displayed brief periods of swaying before beginning stereotypic movements. Initial excitatory movements often included head thrusting upward and stepping motions that progressed into vigorous paddling of the front limbs with extended or kicking hind limbs. These patterned myoclonic movements often began while standing, but were not accompanied by efforts to maintain or restore posture, so that the pig might lurch forward for several steps before losing balance and sliding into lateral recumbency with extended limbs and uninterrupted paddling, or the pig might rear or tip onto its rump with extended hind limbs and appear to be digging frantically in the air until eventually falling over. Pigs did not appear to be responsive to kicks from chamber-mates during this period and rapidly developed fixed, dilated pupils. Since loss of posture was defined by the cessation of head-lifting and dorsal head movement was common during neuromuscular excitation, loss of posture was usually scored after the onset of neuromuscular excitation and upon full lateral recumbency.

There was no significant evidence of an effect of stocking rate on latencies to onset of neuromuscular excitation or last movement after controlling for day of trial and starting body temperature (onset of excitation $P = 0.07$; last movement $P = 0.40$). There was evidence of an effect of stocking rate on latency to loss of posture after controlling for day of trial and starting body temperature ($P = 0.01$). See Table 7 for mean latencies by treatment. There was no significant evidence of an effect of stocking rate on the variability of latency to onset of neuromuscular excitation ($P = 0.10$).

The majority of weaned pigs squealed during euthanasia with argon gas. Squealing typically occurred during the first minute after onset of neuromuscular excitation and manifested at the same time as cessation of voluntary movements and initial signs of cortical disinhibition. Although the timing suggests that squealing during argon euthanasia may not be a genuine indicator of perceived distress, squealing was regarded by many observers as the most disconcerting behaviour associated with argon euthanasia, so the duration of squealing might be considered a relevant index of efficacy. Because squeals from focal pigs could not be isolated or identified reliably from audio and video recordings of groups in the chamber, squealing duration was not compared between treatments in this study.

We quantified the number of potentially offensive contact “strikes” received by focal pigs from conspecifics, such as being kicked, stepped on, or shoved. Nine focal pigs were scored as receiving strikes prior to onset of neuromuscular excitation, with each of those pigs receiving an average of 4 strikes each. See Table 8 for behavioural data by treatment. Pigs had a generally subdued demeanour during the early induction period with minimal interaction despite crowding. No fighting or deliberate piling was observed.

Ten focal pigs, 7 of which were in TRT 1, exhibited pacing or a weight-shifting movement while facing the transparent front panel that resembled an inclination to pace. Space restriction in the chamber from other animals or barrels made turning locomotion difficult for larger pigs in this study, so pacing behaviour was not analysed as a reliable indicator of distress in the chamber. There was evidence of an effect of stocking rate on righting attempt counts after controlling for day of trial and starting body temperature ($P = 0.0003$), with fewer righting attempts by focal pigs at higher stocking rates. There was no significant evidence of an effect of stocking rate on duration of neuromuscular excitation in focal pigs ($P = 0.24$).

Discussion:

Experiment 1

Humane euthanasia should rapidly induce unconsciousness and death (Raj 2006). The measures of efficacy used in this study were latencies to loss of posture, onset of gasping, and last movement, which represent critical transitional points during the induction of unconsciousness and death by hypercapnic hypoxia (Sadler in press). In both age groups, higher stocking rates were associated with higher concentrations of CO_2 at the conclusion of gradual fill, and tests for effect of gas concentration on efficacy variables suggest that this difference was likely a major factor in other significant results observed. Those results included a trend toward negative effect of increased stocking rate on latency to loss of posture in neonates and evidence of negative effects of increased stocking rate on latencies to loss of posture, onset of gasping, and last movement in weaned pigs.
Corroborating the variable loss and recovery of reflexes described for market weight pigs (Holst 2001), young pigs did not lose posture simultaneously when euthanized together in this study. The speed of response varied considerably in some cases, especially within grouped neonates, and was reflective of the wide range of individual characteristics that exist within a single cull selection in the production environment. Increasing the size of a euthanasia group will increase the probability of combining pigs with unusually low and high response rates, risking one or more conscious witness to conspecific distress. However, there was no evidence that stocking rate affected individual variability in latency to loss of posture.

Humane euthanasia should also strive to minimize aversion and distress, so several physiological responses associated with poor welfare were quantified in this study. Ataxia was commonly observed during CO_2 induction of weaned pigs from approximately the onset of hyperpnea until loss of posture. Ataxia during hypercapnia could be caused by impairment of the motor cortex, loss of sensory and proprioceptive signals, or both (Oliver *et al* 1997). Because ataxia occurs prior to complete loss of consciousness, the impaired mobility may be distressing to pigs and thus duration of ataxia was used in this study as a measure of welfare. Hyperpnea, or open-mouth breathing as it appears in pigs, is a form of respiratory compensation in response to decreased pH of blood and cerebrospinal fluid (Gerritzen *et al* 2008; Mota-Rojas *et al* 2012). Humans exposed to carbon dioxide often report an uncomfortable sense of breathlessness and an insatiable drive to breathe more (Llonch *et al* 2012). The prolonged period of hyperpnea observed in both neonatal and weaned pigs may constitute a form of respiratory distress and so was included as a measure of welfare. We did not find evidence of an effect of stocking rate on durations of ataxia or open-mouth breathing, although there was a trend toward decreased duration of open-mouth breathing with increased stocking rate in weaned pigs.

Neuromuscular excitation (including hyperresponsivity, trembling, myoclonus, and convulsions) may be caused by loss of inhibition from the cerebral cortex and rostral reticular formation on the caudal reticular formation (Raj 2006; Llonch *et al* 2012). Neuromuscular excitation was observed consistently in weaned pigs but not in neonates. At first onset, these periods of excitation often coincided with escape attempts and righting attempts, suggesting that pigs retained some degree of consciousness. This conclusion agrees with Raj *et al* (1997) and Rodríguez *et al* (2008), both of which noted muscular excitation in pigs beginning prior to significant changes in brain function when exposed to CO_2. The experience of involuntary excitation while conscious may be distressing to pigs (Dalmau *et al* 2010), so total duration of excitation was included in this study as a measure of welfare. This study found that increased stocking rate was associated with decreased duration of neuromuscular excitation in weaned pigs.

We counted behavioural signs of aversion and distress including pacing, escape attempts, and righting attempts. Urgent pacing along the transparent front panel of the chamber was strongly suggestive of a desire to leave the space. Pacing by pigs often included multiple testing contacts between snout or front hoof and the panel and sometimes rearing and digging behaviours that were counted as potential escape attempts. Pacing occurred in the majority of solitary weaned pigs immediately upon enclosure in the chamber. After a period of pacing, solitary pigs would usually freeze suddenly and back into a corner. Retreat from a noxious stimulus is a characteristic behaviour of pigs (Dalmau *et al* 2010; Llonch *et al* 2012), so this interruption appeared to correspond to first detection of carbon dioxide gas and suggests that pacing was separate from gas aversion. It could be argued that grouped pigs, being more crowded, had neither a clear view of the transparent panel nor an unobstructed path to pace along it; however, paired pigs generally had ample room in the chamber and the incidence of pacing in that treatment was still significantly lower than in solitary pigs.

Escape attempts from a hypercapnic environment may be a reaction to the respiratory discomfort of hyperpnea described above or to direct irritation of mucous membranes by carbon dioxide (Llonch *et al* 2012). Potential escape attempts were observed only in post-weaning age pigs and were least common in the paired focal pigs. The highest potential escape attempt counts were observed in solitary pigs, some of which accrued early during the procedure and may have been related to isolation distress rather than gas aversion. The total incidence of potential escape attempts in grouped focal pigs was similar to that of solitary pigs, although in the case of grouped pigs, the increase over paired pigs may have been caused by the relative averseness of higher CO_2 concentrations (Nowak *et al* 2007; Velarde *et al* 2007; Gregory 2008), since pacing and other signs of early agitation were very rare in the grouped treatment.

Righting attempts were often associated with a sudden change in posture, especially falling or being knocked over, although sleeping neonates would also wake and attempt to stand up at elevated CO_2 levels. Righting attempts, as with ataxia, suggest conscious awareness of a physically impaired state, which may be distressing (Webster & Fletcher 2004). Specifically, righting attempts indicate perception of lost posture and a desire to change posture, presumably due to either discomfort or a desire to be more readily mobile. For these reasons, righting attempt counts were included as a measure of welfare in this study. The effect of increased stocking rate was negative on this behaviour in neonates, but positive on this behaviour in weaned pigs. The reasons for this difference are unclear. One hypothesis, given the high rate at which weaned pigs were shoved and stepped on within the chamber, is that some righting attempts in the older age group were prompted by stimulation from another animal, rather than by an elevated stress level in the chamber.

We did not observe deliberate piling behaviour by any pigs in the chamber, and obstruction of one pig's head by another collapsed pig was rare and transient, so asphyxiation seemed unlikely at these stocking densities. We also did not observe any fighting of pigs in the chamber, despite grouping unfamiliar animals. Swine aggression in lairage usually becomes apparent after about 10 min (Weeks 2008), so if unfamiliar animals must be combined for euthanasia, it may be preferable to do so immediately upon placement in the chamber. Kicks and other potentially offensive strikes received by focal pigs from group-mates were quantified for a descriptive picture of the physical risk associated with confinement and neuromuscular excitation in this species. These events predictably increased with stocking rate and were essentially assured within six-pig groups. When euthanasia cohorts were similarly sized, clonic insults did not appear to be injurious. Idiopathic epistaxis was the only unexpected clinical change occasionally noted in pigs upon removal.

Neonates were considerably less mobile than weaned pigs and more likely to be hypothermic and depressed during enrolment, while weaned pigs were more likely to be emaciated and dehydrated. These differences in health status may have contributed to variations in the results between the two studies, although faster response to the gas by neonates was expected based on the results of Sadler *et al* (2011b). Such hastening by immaturity, along with very small animal size relative to the chamber used, may have made effects of stocking rate more difficult to detect in the neonate age group.

In conclusion, CO_2 gas concentrated fastest in densely stocked chambers, and in weaned pigs this difference was capable of accelerating loss of posture and respiratory arrest and reducing the duration of some aversive states.

This study provided no convincing evidence that isolation during CO_2 gas euthanasia would benefit the welfare of neonatal or post-weaning age pigs. Stocking rate did not appear to be a significant factor in efficacy or welfare of gas euthanasia in neonatal pigs, within the range of one to six animals. Although solitary weaned pigs avoided the frequent kicks and shoves experienced by most grouped pigs at various points during induction, almost all of them exhibited pacing and early escape attempts before gas potency had been established, which lends support to the conclusion that isolation distress outweighs the benefits of seclusion during euthanasia (CCAC 2010). This study also demonstrated improved efficacy and minor welfare benefits from even slightly accelerated concentration of carbon dioxide within the chamber. High frequency of contact in grouped pigs, when combined with faster gas concentration, did not increase the duration of neuromuscular excitation or delay loss of posture. As an investigation of on-farm procedures, our study design did not attempt to separate the effects of density and social interaction, so questions remain regarding the direct impact of group size as a source of comfort or stress during euthanasia of young pigs.

For interpretation of the results by producers, it should be noted that our chamber allowed adequate floor space for lateral recumbency of six average-weight study pigs in our 'high stocking rate' treatment. The welfare implications of exceeding this stocking density, such as by stacking animals in a tall chamber with limited floor space, have never been examined to the authors' knowledge. Ekkel *et al* (2003) looked at space requirements for recumbent pigs when allowing for a voluntary amount of space-sharing and supported using 0.033 X (body weight in kg)$^{0.66}$ as a starting estimate for floor area in square metres, which would equate to about 0.03 m^2 per 1 kg neonatal pig or 0.08 m^2 per 4 kg weaned pig. This formula correlated well with our subjective assessment of a reasonably 'full' chamber during the planning process of this study and may be useful for on-farm estimates of chamber capacity. When euthanizing fewer or unusually small animals, a proportionately reduced chamber volume (via smaller chamber or space-filling) would be expected to provide comparable efficacy without the need to alter timing or flow rate of gradual-fill procedures.

Experiment 2

The onset of neuromuscular excitation and convulsive activity during argon euthanasia signals a release of inhibition on the caudal reticular formation from higher brain centres and can thus be used as a non-invasive approximation of loss of consciousness (Raj 2006). Rapid loss of consciousness is a key criterion of humane euthanasia (AVMA 2013), and variation in latency to loss of consciousness during procedural variations would have major animal welfare implications. This study did not find significant evidence that stocking rate had an effect on latency to onset of neuromuscular excitation, nor did it provide evidence that stocking rate has an effect on latency to last movement, an approximation of the time required to achieve brain death.

Loss of posture and loss of balance have been used in several gas euthanasia studies as a behavioural indicator of unconsciousness (Dalmau *et al* 2010; Llonch *et al* 2012). In this study, grouped pigs tended to lose posture later than solitary pigs, despite little variation in onset of neuromuscular excitation. Loss of posture may not be a reliable index of efficacy for argon gas euthanasia using a prefilled chamber, since the assumption of lateral recumbency was largely dependent on the positioning of the pig at the onset of neuromuscular excitation relative to other animals and objects in the chamber. To the extent that loss of posture can be considered a definitive measure of lost consciousness, then any effect of treatment would be confounded by higher average oxygen exposure values at higher stocking rates, which was most likely caused by the relative amount of chamber manipulation needed to place multiple animals. Excluding the differences in air composition between treatments, interactions between animals that could be responsible for a delay in loss of consciousness, such as strikes or displays of distress behaviour, were not observed in the majority of groups, although less visible factors that were not examined in this study, such as distress pheromones, could be responsible.

The lowered frequency of righting attempts in grouped animals relative to solitary animals may likewise have been the result of crowding in the chamber. Righting attempts most often occurred when pigs fell over from pronounced swaying behaviour immediately prior to the onset of neuromuscular excitation, and visible swaying was rarely observed in grouped pigs. If righting attempts reflected an elevated level of distress and stronger desire to be readily mobile during a gradual loss of control, then we might also expect a delay in latency to loss of posture; however, grouped pigs typically retained posture the longest.

Variation of stocking rate in the range of 1 to 6 pigs did not appear to have a biologically significant effect on the efficacy or welfare indices used in this study for argon gas euthanasia of weaned cull pigs. This study did not provide convincing evidence that isolation during argon gas euthanasia would benefit the welfare of weaned pigs. Achieving a true prefilled environment for euthanasia in a portable chamber is probably not feasible, although the least oxygen would be introduced by rapid placement of a single animal.

Table 1 Scoring guidelines used for clinical signs of illness during study enrolment and codes used to designate reason for euthanasia.

Ocular, nasal, and oral discharge	Dehydration
0 = No discharge	0 = Normal hydration
1 = Serous discharge	1 = Dehydrated
2 = Mucopurulent discharge	**Reason for selection**
3 = Haemorrhagic discharge	1 = Injury or trauma
Activity level	2 = Low viability
0 = Normal activity	3 = Runt
1 = Lethargic	4 = Hernia
2 = Depressed	5 = Scours
3 = Non-ambulatory	6 = Splayed legs
4 = Moribund or unconscious	7 = Other (specified in comments)

Table 2 Guidelines used for body condition scoring during study enrolment (adapted from Straw *et al* 1999).

Score	Pelvic Bones/Tail Head	Loin	Vertebrae	Ribs
1	Pelvic bones very prominent, deep cavity around the tail head	Loin very narrow, sharp edge on transverse spinal process, flank very hollow	Prominent and sharp throughout length of backbone	Individual ribs very prominent
2	Pelvic bones obvious with slight cover; cavity around tail head	Loin narrow, slight cover to edge of transverse spinal process, flank rather hollow	Prominent	Rib cage visible, individual ribs less apparent
3	Pelvic bones covered	Edge of transverse spinal processes covered and rounded	Visible over the shoulder, some cover farther back	Covered but can be easily felt

4	Pelvic bones only felt with firm pressure, no cavity around tail	Edge of transverse spinal processes felt only with firm pressure	Felt only with firm pressure	Rib cage not visible, ribs felt with pressure
5	Pelvic bones impossible to feel, root of tail set deep in surrounding fat	Impossible to feel bones, flank full and rounded	Impossible to feel vertebrae	Not possible to feel ribs
6	Pelvic bones impossible to feel	Thick fat cover	Midline appears as a slight hollow between rolls of fat	Thick fat cover

Table 2 Ethogram used when scoring focal pig behaviour and status from video recordings (adapted from Sadler in press).

Behaviour	Definition
Ataxic movement	An apparent loss of coordination during voluntary movement, such as stumbling, dropped hocks, or crossed-leg stance
Crowded	Pig's head is more than 50% obstructed from free air flow by the presence of another pig/s; or attempts at normal posture, ambulation, or rising from recumbency are restricted by the presence of another pig/s
Neuromuscular excitation	Period of seemingly involuntary excitement with unproductive, repetitive muscular contractions, including trembling, hyperresponsivity, thrashing, head shaking, paddling, or kicking
Onset of gasping	First point at which piglet begins reflexive gasping, characterized by rapid inspiration and gradual expiration at long and possibly irregular intervals, with exaggerated thoracic movements and corresponding sudden opening and slow relaxation of the jaw
Onset of open-mouth breathing	First point at which piglet begins breathing rapidly through continuously open mouth (panting)
Other	Animal behaviour or status does not fit into any other category
Out of view	Animal cannot be seen clearly enough to identify behaviour or status
Pacing	Repetitive, patterned locomotion along an interior wall of the chamber
Potential escape attempt	Apparently voluntary effort to escape the chamber, such as rearing with raised forelegs while pawing at chamber or in air, pushing with head or nose on the chamber lid, or forceful coordinated movement against interior of chamber
Righting attempt	Apparent attempt to restore standing, sitting, or sternal posture from sitting or recumbent position that was unsuccessful in maintaining the posture
Struck	Receives potentially harmful or offensive contact as a result of action by another pig, including being stepped on, bitten, kicked, shoved, or crushed

Table 4 Summary of piglet group mean total body weight (kg) and mean CO_2 concentration (%) at conclusion of gradual fill, with standard error.[1]

	TRT 1	TRT 2	TRT 4	TRT 6
Experiment 1: neonatal pigs in 0.8 m^3 chamber				
total weight (n = 120)	0.8 ± 0.1	1.6 ± 0.1	3.0 ± 0.1	4.9 ± 0.1
CO_2 at 5 min (n = 111)	78.2 ± 0.5	78.2 ± 0.5	79.2 ± 0.5	80.5 ± 0.5
Experiment 2: post-weaning age pigs in 0.25 m^3 chamber				
total weight (n = 90)	5.0 ± 1.4	7.3 ± 1.4	-	24.4 ± 1.4
CO_2 at 5 min (n = 86)	78.5 ± 0.4	79.6 ± 0.4	-	82.6 ± 0.4

[1]Reduced sample sizes reflect software malfunctions during data collection.

Table 5 Latencies to loss of posture, onset of gasping, and last movement in focal pigs, least square means (seconds) with standard error.[2]

	TRT 1	TRT 2	TRT 4	TRT 6
Experiment 1: neonatal pigs				
Loss of posture (n = 120; *P* = 0.06)	83 ± 3.4	84 ± 3.3	85 ± 2.9	76 ± 3.0
Onset of gasping (n = 114; *P* = 0.13)	89 ± 3.5	84 ± 2.6	89 ± 2.7	86 ± 6.2
Last movement (n = 114; *P* = 0.61)	368 ± 15	355 ± 11	390 ± 17	367 ± 13
Experiment 2: post-weaning age pigs				
Loss of posture (n = 90; *P* < 0.01)	107 ± 1.8	109 ± 3.5	-	100 ± 2.1
Onset of gasping (n = 88; *P* < 0.001)	122 ± 3.1	116 ± 3.1	-	111 ± 2.4
Last movement (n = 82; *P* < 0.0001)	488 ± 28	510 ± 29	-	412 ± 15

[2]Reduced sample sizes for onset of gasping reflect focal pigs that did not display gasping or could not be clearly viewed on video. Reduced sample sizes for last movement reflect 14 focal pigs for which values were censored at time of removal from the chamber. P-values are included for linear effect of stocking rate.

Table 6 Behaviours of neonatal focal pigs scored from video in Experiment 1.[3]

	Righting attempts (P)	Righting attempts (C)	Open-mouth breathing (D)
TRT 1	0.43	0.87 ± 0.24 ↑	46 ± 3
TRT 2	0.24	0.41 ± 0.17	44 ± 3
TRT 4	0.31	0.52 ± 0.18	51 ± 3
TRT 6	0.17	0.33 ± 0.21	48 ± 6

[3]Arrows indicate direction of statistically significant positive effect of stocking rate, if any. P = proportion of focal pigs exhibiting behaviour, C = mean count with standard error, D = mean duration in seconds with standard error.

Table 7 Mean latencies to focal pig onset of neuromuscular excitation, loss of posture, and last movement (seconds) with standard error.

	TRT 1	TRT 2	TRT 6
onset of neuromuscular excitation	63 ± 4	62 ± 6	63 ± 4
loss of posture	75 ± 3	77 ± 4	81 ± 4
last movement	264 ± 12	263 ± 15	244 ± 14

Table 8 Behaviours of focal pigs scored from video.[3]

	Righting attempts (P)	Righting attempts (C)	Pacing (P)
TRT 1	0.44	1.32 ± 0.46	0.28
TRT 2	0.46	0.96 ± 0.27	0.08
TRT 6	0.12	0.27 ± 0.17	0.04
	Strikes (P)	**Strikes (C)**	**Neuromuscular excitation (D)**
TRT 1	-	-	55 ± 4
TRT 2	0.13	0.65 ± 0.38	54 ± 3
TRT 6	0.24	0.81 ± 0.37	48 ± 5

[3]P = proportion of focal pigs exhibiting behaviour, C = mean count with standard error, D = mean duration in seconds with standard error.

Figure 1 Typical pattern of oxygen concentration in the chamber. Oxygen concentration (%) values collected during the euthanasia of PG 15 (TRT 1) show a typical pattern of gradual decline during prefill, small peak during pig placement, and re-establishment of critical oxygen level over a total of 16 min (depicted as 96 ten-second time bins).