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Are severely depressed suckling pigs resistant to gas euthanasia?

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Abstract

Severely depressed pigs exhibit differences in a number of important parameters that may affect gas euthanasia, including decreased respiration rate and tidal volume. Hence, the objectives of this study were to assess the efficacy and animal welfare implications of gas euthanasia of suckling pigs with varied disease severity (severely depressed [DP] vs other [OT]). A 2 × 2 factorial design was utilised with two gas types (carbon dioxide [CO_2]; argon [Ar]) and two flow rates (G = gradual, 35% box volume exchange per min [BVE min^{-1}]; P = prefill + 20% BVE min^{-1}). Sixty-two pigs were enrolled and tested as DP/OT pairs in each gas treatment combination. Pigs identified for euthanasia were assigned a subjective depression score (0 = normal to 3 = severely depressed). Pigs scored 3 and ≤ 1 were categorised as DP and OT, respectively. Significantly lower respiration, rectal temperature, pulse and weight were observed for the DP pigs relative to OT. Pigs were assessed for behavioural indicators of efficacy and welfare. No differences were observed between DP and OT when using P-CO_2 or G-CO_2. However in P-Ar, DP had greater latency to loss of consciousness relative to OT (212 [± 22] vs 77 [± 22] s), decreased latency to last limb movement (511 [± 72] vs 816 [± 72] s), greater duration of open-mouth breathing (151 [± 21] vs 69 [± 21] s), decreased duration ataxia (101 [± 42] vs 188 [± 42] s) and decreased righting response (27 [± 11] vs 63 [± 11] s). The G-Ar treatment was removed due to ethical concerns associated with prolonged induction. In conclusion, depression score did not affect pig responses to euthanasia with CO_2 gas, but did affect responses to Ar. Furthermore, Ar was associated with a prolonged euthanasia process, including frequencies and durations of distress behaviours.

Keywords: *animal welfare, argon, carbon dioxide, euthanasia, moribund, swine*

Introduction

Most swine producers and veterinarians agree that euthanasia is the best choice for low viability pigs, especially when there is suffering due to injury or illness. Low viability suckling pigs identified for euthanasia typically consist of two broad categories: unthrifty, ill and depressed pigs vs injured or small but alert pigs. Pigs with low birth weights (< 0.8 kg) are often considered underdeveloped and more than 60% do not survive (Straw *et al* 1999). Carbon dioxide has been identified as an acceptable inhalant method for euthanasia of pigs because it is a rapid depressant with established analgesic and anaesthetic properties (AVMA 2013). Carbon dioxide is commonly used for stunning market-weight pigs at slaughter, and remains the most commonly implemented gas for on-farm euthanasia of suckling and nursery age pigs in the USA (Daniels 2010). The American Veterinary Medical Association Panel on Euthanasia notes:

> … parameters of the technique need to be optimized and published to ensure consistency and repeatability. In particular, the needs of piglets with low tidal volume must be explored (AVMA 2013; p 61).

Additionally, anecdotal reports from stockpeople suggest efficacy is decreased when euthanising the moribund (severely depressed) pig relative to a more robust and alert pig, and this may account for failed euthanasia attempts in which additional exposure to the gas or a secondary euthanasia method is required.

Severely depressed pigs differ from robust pigs in several physiological parameters that may be important for gas euthanasia. Several causal factors could contribute to creating the depressed state, including disease, injury and

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underdevelopment. These pigs tend to have low respiration rates and tidal volumes (AVMA 2013; p 61). This would lead to decreased total volume exchange rates of gases into and out of the body (Guyton & Hall 2010). There are a number of factors following birth that may contribute to decreased survival, including greater latency to udder contact, greater latency to colostral intake and a greater than average decrease in temperature post-birth (Straw *et al* 1999). These low birth-weight pigs are often in a state of severe respiratory acidosis (Straw *et al* 1999). Furthermore, severely depressed pigs are likely hypoglycaemic, contributing to a variety of symptoms observed including low temperature, convulsions and comatose state (Straw *et al* 1999).

Carbon dioxide is mildly acidic, causing irritation to the mucus membranes in humans (Danneman *et al* 1997), leading to questions regarding the humaneness of this gas for pig euthanasia (Wright *et al* 2009). Argon has been proposed as an alternative inhalant in slaughter facilities for stunning and killing pigs to improve animal welfare (Raj 1999). Argon is a noble gas, and as such is likely unreactive throughout the physiological systems (Mann *et al* 1997). Hence, loss of consciousness and death are produced through hypoxia, creating the physiological state of hypocapnic anoxia (Raj 1999). According to the AVMA (2013), argon is considered conditionally acceptable as a euthanasia inhalant for swine. The European Food Safety Authority states that although gas euthanasia requires sophisticated equipment, this technology has been identified as having high potential for humane stunning and killing of animals (EFSA 2004). Furthermore, EFSA recommends the use of noble gases such as argon that induce unconsciousness through hypoxia rather than hypercapnia. Controlled atmospheric killing with argon gas is used in some commercial broiler processing facilities, and since 2002, animal protection organisations such as People for the Ethical Treatment of Animals (PETA 2002) and the Humane Society of the United States (HSUS 2009) have encouraged retailers to source their chicken meat from companies using this technology. Both AVMA and EFSA acknowledge the need for further research to identify best management practices for preferred gas mixtures and methods of application. Since the physiologic effects of these gases differ, it is important that both carbon dioxide and argon be examined in relation to the severely depressed pig.

Euthanasia is comprised of two stages: (i) induction of unconsciousness (insensibility); and (ii) death. It is the induction phase that is critical to the welfare of the pigs. Duration of the entire process, including death, is important to ensure practical implementation. Pain and distress are affective states, and hence can only be measured indirectly in humans and animals. Humans report feelings of pain and distress when exposed to carbon dioxide (Gregory *et al* 1990). Distress associated with carbon dioxide has been assessed in pigs using behavioural responses, such as escape attempts, hyperventilation, sneezing, coughing, head shaking and vocalisations (Dodman 1977; Raj & Gregory 1996; Velarde *et al* 2007; Rodríguez *et al* 2008; Sadler *et al* 2014). Although differences in behaviour are observed during induction of insensibility, it is difficult to ascertain whether these are accurate indicators of distress since these behaviours may coincide with the induction process or when insensibility has begun. Raj and colleagues (1997) found loss of somatosensory-evoked potentials, indicative of brain responsiveness, occurred within 21 s of exposure to 90% carbon dioxide and hence, signs of moderate to severe respiratory distress (coughing, open-mouth breathing, squealing) occurring during this period are likely associated with conscious awareness, in the grower pig (40 kg). Similarly, Rodríguez and colleagues (2008) concluded that in the grower pig (25–35 kg) loss of consciousness occurred, on average, 60 s after exposure to 90% carbon dioxide on the basis of middle latency auditory-evoked potentials and that prior excitatory movements (lateral head movement, sneezing, vocalisation) were conscious movements associated with aversion.

In contrast with these recommendations, our previous research suggests decreased welfare during induction of unconsciousness when pigs were stunned with 100% argon relative to 100% carbon dioxide applied at 35% box volume exchange per min (BVE min^{-1}). Argon was associated with increased latency to loss of posture, increased duration of open-mouth breathing and distress calls (Sadler 2013). However, efficacy of 100% argon at this flow rate for euthanasia vs stunning has not been examined. Rault and colleagues (2013) examined argon as the first step in a two-phase gas euthanasia process for suckling pigs, but efficacy of argon gas as a single gas method for pig euthanasia has not been examined.

The primary objective of this research was to evaluate pigs in a severely depressed state compared to pigs that required euthanasia but were not classified as depressed. A second objective was to compare the effects of CO_2 and argon as single-gas methods for piglet euthanasia. Third, two flow rates (prefill vs gradual) were compared within a gas-type treatment.

Materials and methods

Experimental design

Suckling pigs identified for euthanasia were allocated to one of two disease status categories: DP = severely depressed; and OT = other. Effects of each disease status were assessed in a 2 × 2 factorial design with two gas types (CO_2 = 100% carbon dioxide; Ar = 100% argon) and two flow rates (G = gradual fill at 35% BVE min^{-1}; P = prefill + 20% BVE min^{-1}). The experiment was designed to utilise eleven DP/OT pairs for each gas treatment combination. This design would utilise 88 pigs (2 disease statuses × 2 gases × 2 flow rates × 11 reps per gas treatment). Gas treatments were run in a randomised sequence. The American Veterinary Medical Association notes pigs can be euthanised either with gradual displacement or by introducing the pigs into a pre-filled environment (AMVA 2013; p 60). On the basis of previous research in our laboratory (Sadler *et al* 2014), gradual flow rate in this experiment utilised a 35% BVE min^{-1}. On-farm, prefill is currently the most commonly implemented flow rate (L Sadler, personal observation 2013), and thus it was of high priority to examine its efficacy.

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Study animals and enrolment criteria

Pigs were sourced and housed from a commercial sow farm, and genetics were a Landrace × Yorkshire cross × Duroc sire line. Pigs were eligible for enrolment if they were less than 21 days of age, and were identified by farm staff as low viability or injured and in need of euthanasia. These pigs were placed in a cart with wood-shavings and a heat lamp and placed in the test room. Pigs were assigned a subjective depression score (FDA 2007) by a single technician. The depression score ranged from zero to three (0 = Normal; alert, active, normal appetite, well-hydrated, normal coat; 1 = Mild; moves slower than normal, slightly rough coat, may appear lethargic but upon stimulation appears normal; 2 = Moderate; inactive, may be recumbent but is able to stand, gaunt, may be dehydrated; 3 = Severe; down or reluctant to get up, gauntness evident, dehydrated). Based on this four-point scale, pigs were placed into a disease category (3 = DP; 0 or 1 = OT); pigs that scored a 2 were excluded from this study. Individual pigs were then randomly placed into DP/OT pairs. Pig pairs were marked with an animal safe marker (LA-CO Industries, Elk Grove, IL, USA).

Euthanasia equipment

Gas was administered to the pigs via a modified Euthanex AgPro™ system (V-ast, Mason City, IA, USA). This gas delivery apparatus was designed by Euthanex Corporation (Palmer, PA, USA), a manufacturer of gas delivery systems for rodents and small animals. To facilitate behavioural observation, the box was constructed of clear plastic on the top and front panels. The top panel was hinged for placing pigs into the box, with an airtight foam seal. The remaining four panels were constructed of opaque plastic. The inside dimensions of the box measured 60 × 43 × 60 cm (length × width × height). The floor was fitted with a rubber mat (Rubber floor mats, Kraco Enterprises, LLC, Compton, CA, USA) and a layer of wood sawdust (~2 cm in depth; TLC Premium Horse Bedding, Centerville, AR, USA) to aid in traction and comfort for the pigs.

The box had two 0.64-cm inlet valves located on one panel 12.70 (CO_2) and 22.86 cm (Ar) from the side and 3.81 cm from the top. The gas flowed through rubber hoses that were 3.25 m in length and 0.64 cm in diameter prior to entering the box. A 0.95-cm outlet valve was located on the opposite panel from the inlet valves, 30.48 cm from the side and 6.35 cm from top, and was vented outdoors for worker safety. Constant and precise gas flow was provided by compressed gas cylinders equipped with compressed gas regulators and meters (Western Enterprises, Westlake, OH, USA). The CO_2 gas was industrial grade (99% pure). The Ar had a guaranteed analysis of 99.99% pure. To produce the pre-filled environment, gas was supplied to the closed box at 50% BVE min^{-1} for 5 min. This procedure was previously shown to produce an atmosphere within the box of $< 2\%$ O_2 at the head level of a standing pig (Sadler 2013). Prior to each treatment, sawdust was removed from the box by a vacuum (Shop Vac 10 Gallon Ultra Pro Vacuum, 185 CFM, Willamsport, PA, USA), a clean rubber mat was placed in the box, and fresh sawdust was provided. The vacuum was also utilised to remove gas traces, pulling air from the bottom of box for a minimum of 3 min.

Environmental conditions

A HOBO data logger (U23-001, Onset Computer Corporation, Cape Cod, MS, USA) was used to record temperature (°C) and relative humidity (%) within the box. The data logger was set to record every 10 s. Oxygen concentrations (%) were collected every second at pig level with an oxygen sensor (TR25OZ, CO2Meter.com, Ormond Beach, FL, USA) attached to a HOBO data logger (U12, Onset Computer Corporation, Cape Cod, MS, USA). Data were exported into Microsoft Office Excel® (version 2007, Redmond, WA, USA). A CO_2 meter (CO2IR-WR 100%, CO2Meter.com, Ormond Beach, FL, USA) monitored concentrations every 1.25 s. However, due to technical difficulties these data were not reported.

Euthanasia procedure, confirmation of insensibility and death

On the testing day, vital signs (respiration, rectal temperature, pulse and weight) were collected for all pigs prior to placement in the box. Pigs were euthanised within 4 h of being identified by farm staff for euthanasia. The testing room provided isolation, minimising noise and distractions. Pig pairs (DP/OT) were placed into the box in a standing posture, and gas was immediately applied until the pigs were confirmed dead. One of two observers was randomly assigned to a pig, performing all tests for signs of insensibility and behavioural observations. Two minutes following respiratory arrest, pigs were removed individually from the box and checked for signs of insensibility (Whelan & Flecknell 1992; Kissin 2000; National Pork Board 2009; Grandin 2010). Three insensibility tests were conducted: (i) corneal reflex response, in which the eye was touched with the tip of a finger for absence of an eye blink or withdrawal response; (ii) pupillary reflex, in which a light-beam (Mini MAGLite, Mag Instrument Inc, Ontario, CA, USA) was shone into the eye for absence of pupil constriction; and (iii) nose prick, in which a 20-gauge needle was touched to the snout distal to the rostral bone for absence of a withdrawal response. After insensibility was confirmed, cardiac arrest was confirmed by auscultation. If signs of sensibility or cardiac activity were present, the pig was placed back into the box for an additional minute of gas exposure. This process was repeated until confirmation of cardiac arrest to establish duration of dwell time necessary for death. The signs of insensibility verification process required < 15 s, including time to remove and return the pig to the box. Auscultation was conducted for > 30 s, with piglets immediately placed back in the box if a heartbeat was detected.

For ethical and practical reasons, the protocol was terminated if pigs displayed signs of consciousness (retained posture, making righting attempts, vocalisations, or had not transitioned to gasping) after 10 min of gas exposure. Gasping, an indicator in disruption of the ventral respiratory group, was defined as rhythmic breaths characterised by very prominent and deep thoracic movements. Additionally, a ceiling value of 15 min was used for death (cardiac arrest) after loss of consciousness. For pigs that did not achieve these measures in the designated time, manual blunt force trauma (National Pork Board 2009) was applied as a secondary euthanasia method.

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Table 1 Ethogram developed for investigating latency (L), duration (D), prevalence (P) or frequency (F) of behavioural indicators of distress or sensation during euthanasia.

Behaviours	Definition
Open-mouth breathing† (D, P)	Upper and lower jaw being held open with the top lip pulled back, exposing gums or teeth and panting (pronounced inhalation and exhalation and exhalation observed at the flanks)[1, 2]
Ataxia† (D, P)	Pig is moving in a seemingly unco-ordinated fashion; lack of muscle co-ordination during voluntary movements[3]
Righting response† (D, P, F)	Pig is making attempt to maintain either a standing or lying sternal posture but is not successful in maintaining the position, seemingly co-ordinated movements. The event was defined as each time effort was made and the muscles relaxed
Out of view† (D)	Pig could not be seen clearly enough to identify the behaviour or posture; or animal was removed from box
Oral discharge‡ (P)	Fluid discharge coming from mouth may be clear and fluid, viscous or blood. Type of discharge was noted
Nasal discharge‡ (P)	Discharge from the nasal cavity, may be clear and fluid, viscous or blood. Type of discharge was noted
Ocular orbit discharge‡ (P)	Discharge from the ocular orbit, may be clear and fluid, viscous or blood. Type of discharge was noted
Vomiting‡ (P)	Ejection of gastrointestinal contents through the mouth[4]
Escape attempt bout† (P)	Pig is raising their forelegs on the side of the wall of the box or pushing quickly and forcefully with their head or nose on the lid of the box; forceful co-ordinated movement against the exterior of the box; occurrences within a 10-s period will be scored as a single bout[5]
Loss of consciousness† (L)	Pig had loss of posture; pig is slumped down, making no attempt to right itself, may follow a period of attempts to maintain posture, loss of attitude of position of the body[1,5]; no vocalisations; pig is gasping; rhythmic breaths characterised by very prominent and deep thoracic movements, with long latency, may involve stretching of the neck
Last limb movement† (L)	No movement is observed in the pig's limbs
Respiratory arrest† (L)	No thoracic movement visible verified for 1-min duration
Cardiac arrest† (L)	No cardiac activity confirmed by auscultation, verified for 30-s duration

[1] Adapted from Velarde *et al* 2007; [2] Adapted from Johnson *et al* 2010; [3] Adapted from Blood *et al* 2007; p 150; [4] Adapted from Hurnik *et al* 1985; [5] Adapted from Raj & Gregory 1996;
† Denotes associated parameter was collected through video observation; ‡ Denotes associated parameter was collected through live observation.

Modification to original study design due to ethical concerns

In this study, 60% of the pigs in the Ar treatments required a secondary euthanasia method (1 pig G-Ar; 9 pigs P-Ar). Of these, 73% displayed signs of sensibility after 10 min. Due to ethical concerns regarding this high proportion of pigs requiring a secondary euthanasia step, G-Ar was terminated after two repetitions (two pig pairs) and P-Ar was dropped after seven repetitions (seven pig pairs). This resulted in a reduced number of enrolled pigs compared to the study design; a total of 62 pigs were enrolled. Thus G-Ar (n = 2) was dropped from the statistical analysis, and sample size was reduced for P-Ar (n = 7). In the first run of P-Ar, the originally designed protocol was followed, using 20% BVE min^{-1} following pig placement in the box, however in an effort to increase success for all other subsequent Ar runs, gas was applied at 50% BVE min^{-1}. This was done to ensure low oxygen concentrations were re-established, after placement of the pigs in the box, as quickly as possible.

Behavioural observations

Behavioural data were collected directly and via video recording. For direct observation, each observer sat approximately 1.5 m from the box and recorded behavioural indicators of distress, physiological responses and insensibility (Table 1). Video recordings were recorded utilising a Noldus Portable Lab (Noldus Information Technology, Wageningen, The Netherlands). Two colour Panasonic cameras (WV-CP484, Kadoma, Japan) were connected to a multiplexer, allowing the image to be recorded onto a PC using HandiAvi (v4.3, Anderson's AZcendant Software, Tempe, AZ, USA) at 30 frames s^{-1}. Behavioural data from video were collected by a single trained observer, blind to disease status and treatments, using Observer® software (v10.1.548, Noldus Information Technology). Data were collected for each individual pig for behavioural and physiological indicators of distress and efficacy of the euthanasia process (Table 1). Latencies for all behaviours were determined from the point when each pig was placed into the box.

Assessment of lungs

Immediately upon confirmation of death, necropsy was performed. Lungs were removed and scored by a single technician, blinded to disease status, for total macroscopic lung lesions as described by Opriessnig and colleagues (2004). The scoring system is based on gross visible damage and the approximate volume each lung lobe contributes to the whole lung: the right cranial lobe, right middle lobe, cranial part of the left cranial lobe and caudal part of the left cranial lobe contribute 10% each to total lung volume, the accessory lobe contributes 5% and the right and left caudal

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Table 2 Mean (± SEM) of descriptive parameters prior to euthanasia for cull suckling pigs classified as severely depressed or other.

Parameter	Mean (± SEM) depressed (n = 31)	Mean (± SEM) other (n = 31)	*P*-value
Respiration rate (per 10 s)	8 (± 2)	11 (± 2)	0.0430
Pulse rate (per 10 s)	24 (± 3)	32 (± 3)	< 0.0001
Temperature < 31.7°C[†] (number of pigs)	22 (N/E)	3 (N/E)	N/E
Temperature if > 31.7°C[‡]	35.9 (± 0.3)	38.3 (± 0.3)	0.0236
Weight (kg)	1.0 (± 0.3)	1.6 (± 0.3)	0.0125
> 0.8 kg (number of pigs)	15 (N/E)	8 (N/E)	N/E
Female (number of pigs)	15 (N/E)	16 (N/E)	N/E
Male (number of pigs)	16 (N/E)	15 (N/E)	N/E
Total lung damage (%)	20 (± 6)	10 (± 6)	0.2498

N/E = value not estimated;
[†] Thermometer utilised was not capable of recording temperatures below 31.67°C;
[‡] For estimates of temperature if > 31.67°C.

lobes contribute 27.5% each. Each lobe was scored as follows: 0% = no gross damage; 50% = some damage, with ≤ 50% of the lobe grossly affected; 100% = > 50% of the lobe grossly affected. These lobe scores were aggregated for a total lung score, ranging from 0–100% affected.

Samples of the lung tissue were collected, with diseased tissue sampled when grossly visible. If no gross lesions were visible, two samples were collected from each of the left and right middle lobes. Samples were collected and fixed in 10% buffered formalin until scored. Histological examination was performed by pathologists at the Iowa State University Veterinary Diagnostic Laboratory (VDL), who were blind to disease status and gas treatments. Sections of formalin-fixed lung were embedded in paraffin, processed per the VDL protocol and stained with haematoxylin and eosin stains. A pathologist examined lung sections for evidence of ante mortem haemorrhage or atelectasis and also characterised the lesions of pneumonia as non-suppurative interstitial pneumonia or suppurative bronchopneumonia. Pleuritis, when present, was also noted.

Statistical analysis

Behaviours were quantified as latency, duration, percent of pigs displaying or number of occurrences as indicated for the parameter. Data were analysed using linear mixed models fitted with the GLIMMIX procedure (duration and frequency; SAS Inst Inc, Cary, NC, USA) or with a Cox proportional hazard model fitted with the PHREG procedure (latency) of SAS. Individual pig was the measurement unit for depression score whereas pig pair served as the experimental unit for gas treatments. Least square means' estimates for each treatment group and the corresponding standard errors (± SEM) are reported. The linear model included the fixed effect of disease status (DP/OT) and gas treatment (P-CO_2, G-CO_2, P-Ar) and all two-way interactions. A random blocking effect of pig pair was included. The Kenward-Rogers method was utilised for determining the denominator degrees of freedom. Statistical significance was established at $P \leq 0.05$.

Protocol

The Iowa State University Institutional Animal Care and Use Committee and the Environmental Health and Safety Office approved the protocol for this experiment.

Results

At enrolment, pigs in the DP group had lower respiration rates, lower body temperatures and lower weights relative to OT (Table 2). Pulse, respiration and weight were examined as covariates for all measures of efficacy (loss of consciousness, last limb movement, respiratory arrest, cardiac arrest) and did not differ ($P > 0.10$). Light pigs (weighing < 0.8 kg) were examined relative to heavier pigs across all treatments, while controlling for disease status. Light pigs had shorter latencies to respiratory arrest (–252 s, $P = 0.0272$) and cardiac arrest (–306 s, $P = 0.0261$). Differences were not observed by weight category for loss of consciousness or last limb movement.

As assessed during necropsy, total lung damage did not differ between DP and OT pigs (Table 2). This gross assessment also indicated there was minimal lung damage in this population of pigs. Histological examination confirmed gross lesion scoring, indicating haemorrhages, atelectasis or lesions in all but four pigs identified as having gross lesions. Additionally, all pigs identified grossly as having healthy lungs lacked histological indicators of damage.

Latency to loss of consciousness, last limb movement, respiratory arrest and cardiac arrest did not differ between DP and OT pigs in either P-CO_2 or G-CO_2 (Table 3). In P-Ar, latency to loss of consciousness was almost three-fold longer ($P = 0.001$) for DP compared to OT; whereas, latency to last limb movement was shorter ($P = 0.004$) for the DP pigs than OT. However, no differences were observed between DP and OT pigs for respiratory arrest or cardiac arrest in the P-Ar gas treatment. Comparing gas treatments, independent of disease status, latency to loss of consciousness was shortest in P-CO_2

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Table 3 Parameters of efficacy of gas euthanasia comparing disease status of suckling pigs within gas treatments. Means are based on non-zero values.

	Prefill CO_2		Gradual CO_2		Prefill Ar	
Parameter	**Depressed (n = 11)**	**Other (n = 11)**	**Depressed (n = 11)**	**Other (n = 11)**	**Depressed (n = 6)**	**Other (n = 6)**
Loss of consciousness	37 (± 22)^{a}	40 (± 22)^{a}	99 (± 21)^{b}	97 (± 21)^{b}	212 (± 32)^{c}	77 (± 29)^{a,b}
Last limb movement	142 (± 53)^{a}	167 (± 53)^{a}	289 (± 51)^{b}	322 (± 51)^{b}	511 (± 72)^{c}	816 (± 72)^{c}
Respiration arrest	377 (± 80)^{a}	400 (± 80)^{a}	503 (± 55)^{b}	388 (± 55)^{a}	741 (± 223)^{c}	1,233 (± 223)^{c}
Cardiac arrest	780 (± 93)^{a}	828 (± 93)^{a}	748 (± 89)^{a}	736 (± 89)^{a}	907 (± 125)^{a}	1,329 (± 125)^{a}

$^{a–c}$ Within a row, least square means (± SEM) lacking a common superscript letter differ, $P < 0.05$.

Table 4 Mean (± SEM) durations (s) of behavioural and physiological measures of distress for suckling pigs of different disease status within gas treatment. Means are based on non-zero values.

	Prefill CO_2		Gradual CO_2		Prefill Ar	
Parameter	**Depressed (n = 11)**	**Other (n = 11)**	**Depressed (n = 11)**	**Other (n = 11)**	**Depressed (n = 6)**	**Other (n = 6)**
Open-mouth breathing	14 (± 15)^{a}	21 (± 15)^{a}	35 (± 15)^{a}	29 (± 15)^{a}	151 (± 21)^{b}	69 (± 21)^{a}
Ataxia	12 (± 31)^{a}	16 (± 31)^{a}	35 (± 29)^{a}	35 (± 29)^{a}	101 (± 42)^{b}	188 (± 42)^{c}
Righting response	13 (± 8)^{a}	3 (± 8)^{a}	20 (± 8)^{a}	10 (± 8)^{a}	27 (± 11)^{a}	63 (± 11)^{b}

$^{a–c}$ Within a row, least square means (± SEM) lacking a common superscript letter differ, $P < 0.05$.

(P-CO_2 vs G-CO_2, $P = 0.0219$; P-CO_2 vs P-Ar, $P = 0.0015$), whereas G-CO_2 and P-Ar did not differ. Similarly, latency to last limb movement was shortest in P-CO_2 (P-CO_2 vs G-CO_2, $P = 0.0052$; P-CO_2 vs P-Ar, $P < 0.0001$), and was twice as long in P-Ar relative to G-CO_2 ($P < 0.0001$). Latency to respiratory arrest did not differ between P-CO_2 and G-CO_2, and both were shorter than P-Ar (P-CO_2 vs P-Ar, $P = 0.0008$; G-CO_2 vs P-Ar, $P = 0.0016$). Latency to cardiac arrest did not differ between gas treatments.

All pigs displayed open-mouth breathing and ataxia (Table 4). Open-mouth breathing did not differ between DP vs OT pigs in either P-CO_2 or G-CO_2. In P-Ar, duration of open-mouth breathing was twice as long ($P = 0.0035$) for DP relative to OT. Similarly, duration of ataxia did not differ between DP compared to OT pigs in either P-CO_2 or G-CO_2, but in P-Ar, duration of ataxia was shorter ($P = 0.037$) for DP compared to OT. Proportion of pigs displaying a righting response did not differ between DP vs OT pigs (DP P-CO_2 = 55%, OT P-CO_2 = 27%; DP G-CO_2 = 64%, OT G-CO_2 = 64%; DP P-Ar = 83%, OT P-Ar = 100%). When it was observed, duration of the righting response did not differ between DP vs OT pigs in either P-CO_2 or G-CO_2 (Table 4). In P-Ar, duration of righting response was half as long ($P = 0.0030$) in DP than OT pigs. The number of efforts made during the righting response by a single pig ranged from zero to 19. Number of efforts did not differ between DP and OT pigs in P-CO_2 (mean [± SEM] number of events: DP = 3.4 [± 1.3]; OT = 0.7 [± 1.3]), or in G-CO_2 (DP = 3.4 [± 1.2]; OT = 2.4 [± 1.2]). In P-Ar, fewer righting response efforts were observed for DP compared to OT (DP = 4.3 [± 1.7]; OT = 11.8 [± 1.7]; $P = 0.0030$).

In all gas treatments, proportions of pigs displaying escape attempts did not differ between DP compared to OT pigs, and were rare. Escape was displayed by one OT pig in G-CO_2 and by three DP pigs and three OT pigs in P-Ar. All four pigs in G-Ar made escape attempts. On a prevalence basis, regardless of gas treatment, this equates to 19 OT vs 16% DP pigs; whereas, by gas type this is 2 vs 56% for CO_2 and Ar, respectively. Oral discharge was also a rare event, observed in one OT pig in P-CO_2, one OT pig in G-CO_2 and two DP pigs in P-Ar. Ocular discharge was only observed in two OT pigs in P-CO_2, and nasal discharge was observed in one OT pig in P-CO_2 and one DP pig in P-Ar. Vomiting and sneezing were not observed. Out of view was scored for less than 1% of the total observation for any individual pig.

Comparing gas treatments, independent of disease status, differences were not observed between P-CO_2 and G-CO_2 for duration of ataxia, open-mouth breathing or righting response. Greater duration of ataxia was observed in P-Ar relative to P-CO_2 ($P = 0.0436$) but did not differ relative to G-CO_2 ($P > 0.1$). Similarly, greater duration of open-mouth breathing was observed in P-Ar relative to both P-CO_2 ($P = 0.0026$) and G-CO_2 ($P = 0.0129$). P-Ar was also associated with greater proportion of pigs displaying a righting response and greater duration compared to P-CO_2 ($P = 0.0005$) and G-CO_2 ($P = 0.0037$). A greater number of righting (response) efforts was also observed in P-Ar compared to P-CO_2 ($P = 0.0002$) and G-CO_2 ($P = 0.0009$). Differences in the righting response were not observed between the CO_2 treatments.

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Over all days, the average temperature inside the box was 19.9°C, ranging from 16.4 to 22.8°C. Relative humidity averaged 50.9% and ranged from 31.2 to 83.4%. Over all trials, initial O_2 concentrations were 2–8, 21 and 5–7% for P-CO_2, G-CO_2 and P-Ar, respectively. The designed protocol required the lid to be opened for placement of pigs for P flow rates, and for removal of pigs to confirm insensibility. Both CO_2 and Ar are heavier than atmospheric air and it was expected modified gas concentrations would stay relatively constant. However, the process of checking for insensibility made maintaining continuous O_2 concentrations below 3% difficult. Although gas was flowing the entire time, opening the lid resulted in increased O_2 concentrations (< 7%) in both the CO_2 and Ar treatments. Oxygen concentrations < 3% were regained in less than 45 s. Latency to return to the initial O_2 concentration < 3% was not numerically longer for the single treatment of P-Ar followed by 20% relative to longest 50% P-Ar latency for this parameter. For G-CO_2, pigs lost posture when O_2 concentrations were 8–16%. As P-CO_2 and P-Ar were pre-filled by definition, pigs lost posture at < 7% O_2 concentrations.

Discussion

In the current study, pigs classified as DP or OT did not differ in behavioural and physiological responses associated with efficacy or distress when euthanised using P-CO_2 or G-CO_2. However, with a small sample size, euthanasia of DP pigs took longer and resulted in differences for distress indicators when utilising P-Ar. Additionally, Ar resulted in behaviour and physiologic responses that raise concerns about efficacy and welfare for all pigs euthanised with Ar, regardless of flow rate or disease status.

The subjective categorisation of pigs into DP and OT disease categories, performed by behavioural scoring of depression, was validated since the subsequent vital parameters indicated pigs classified as DP had a higher compromised health status relative to the OT pigs. Although lung lesions were not different, respiratory rates were lower in the DP pig, which could directly affect the exchange of gas through the respiratory system. The similar lesion scores would indicate any observed differences were not a result of limitations to physical exchange of gas or compromise of the lungs.

Our objective for this study was to assess efficacy and distress of euthanasia procedures using an experiment designed to simulate on-farm conditions. Although more invasive or laborious methods to assess efficacy and distress, such as EEG or ECG monitoring, can provide robust data in the laboratory, they were not practical on-farm and could not be used in tandem with measurement of naturally occurring behaviours induced during gas euthanasia procedures. Behaviour was chosen as the primary outcome of interest for distress since behavioural observations provide more sensitive measures of the animal's experience than physiologic responses, particularly since euthanasia with inhalant gases can produce confounding effects on physiologic responses (Burkholder *et al* 2010). Pigs were tested as pairs (DP/OT) to reduce potential noise in the observed behavioural responses due to social isolation. Although vocal and physical stimulation between the two pigs has the potential to alter the process, stocking density was not found to affect behavioural responses of suckling pigs during gas euthanasia when pigs were placed in the box singly or in groups of 2, 4 or 6 pigs (Fiedler *et al* 2014). Conversely, stocking density during gas euthanasia significantly affected the responses of weaned pigs, with solitary weaned pigs displaying significantly greater distress behaviours and latencies to loss of posture when compared to pigs euthanised in groups. Since group size was confounded with gas concentration, further research is needed to clarify the potential effects of multiple pigs placed in a single box during gas euthanasia.

Efficacy — disease status

We examined four different behavioural and physiological indicators of efficacy (loss of consciousness, last limb movement, respiratory arrest, cardiac arrest; Table 3). All four of these measures indicated that disease status of the pig, as defined in this study, was not a predicting factor for determining efficiency in P-CO_2 or G-CO_2. The results of this study contradict the current AVMA euthanasia guidelines, which note an incapacitated pig "...will not die as rapidly as larger more viable pigs" (AVMA 2013; p 61).

The first assessed indicator of efficacy was loss of consciousness. In our experiment, the transition from consciousness to unconsciousness was determined in part with loss of posture, which has been identified in previous research as an indicator of loss of consciousness (Forslid 1987; Raj & Gregory 1996; Velarde *et al* 2007). When using P-Ar, the DP pigs took approximately two times longer than OT to reach loss of consciousness, but were quicker to achieve last limb movement. The different physiologic effects of CO_2 compared to Ar may explain the increased latency to loss of consciousness. The use of CO_2 creates a hypercapnic state (Raj *et al* 1997) and affects multiple body systems due to decrease in pH, including in the blood and interstitial fluid, which may create a similar euthanasia process for animals regardless of disease status. In part, this may be due to the possibility that the DP pig may be in an acidotic state at the time of euthanasia (Straw *et al* 1999). In contrast, Ar creates a hypoxic state, and will make euthanasia more difficult for diseased pigs with compromised lung function. Further studies are necessary to understand the physiological mechanisms of this observation.

Efficacy — gas treatment

When examining gas treatments, latency to loss of consciousness was 2.6× longer for G-CO_2 relative to P-CO_2, whereas P-Ar took 3.8× longer than P-CO_2. These results are in sharp contrast to Raj (1999), who found latency to loss of consciousness was not affected by gas type when finisher pigs were exposed to 90% Ar or 80–90% CO_2. Additionally, latencies to loss of consciousness (15 and 18 s for 90% Ar and 80–90% CO_2, respectively) in Raj (1999) were considerably shorter than observed in our study. It is surprising that 90% Ar, with no known effect on the body, was capable of producing loss of consciousness through hypoxia in less than 20 s. This time-frame is almost 4× less

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than that observed for OT pigs and 10× less than that observed in DP pigs exposed to Ar in our study. The differences between studies may be due to age or weight of the pigs. Another factor may be the method of gas application; in the current experiment, opening the chamber lid to place pigs inside allowed some reintroduction of atmospheric air. When utilising gas to stun prior to slaughter, pigs are lowered into a pit where maintaining a constant modified atmosphere is more feasible. Additionally, the method utilised to confirm efficacy (opening the lid) may have amplified the observed differences, as the Ar treatment with increased latencies between last movement and confirmation of death were exposed to a greater number of increased oxygen events. As well, in the current study pigs were exposed to normal atmospheric air for up to 45 s during the checks for signs of insensibility and death. However, this exposure occurred after respiratory arrest was visually confirmed, and thus gas exchange into and out of the pig's body would have been minimal. The findings in the current study are similar to the pattern observed in swine of similar age (Sutherland 2011). Sutherland (2011) established that transitional EEG occurred at 33 and 61 s after exposure to 100% CO_2 and Ar, respectively, which is considered incompatible with consciousness (Blackmore & Delany 1988), and isotonic EEG (undisputed loss of awareness) occurred at 46 and 69 s, respectively. Therefore, exposure to 100% Ar appears to double the latency to unconsciousness in young pigs as compared to 100% CO_2.

Identification of expected last limb movement during gas euthanasia is important for stockpeople to recognise when the process is not occurring within acceptable guidelines and intervention is necessary. It also serves as a general indicator of efficacy of the process. In this study, use of G-CO_2 and P-Ar prolonged the euthanasia process by 2 and 4×, respectively, relative to P-CO_2. Hence, this parameter provides further evidence that Ar decreases efficiency of gas euthanasia.

Regular breathing, including open-mouth breathing, is controlled by the ventral respiratory group (Guyton & Hall 2010). When this system fails, gasping is recruited (St John 2009). Respiratory arrest (cessation of gasping) represents the point at which gases can no longer be introduced into the pig's respiratory system. This point is critical to the euthanasia process because the pig will not recover without intervention. During gas euthanasia, gasping will become slower and shallow until breathing finally ceases. In this study, respiratory arrest was the last movement by the pig that was observed, which is consistent with observations conducted using mink (Hansen *et al* 1991), perhaps indicating the death process during gas euthanasia is conserved across mammals. Surprisingly, even though latencies to loss of consciousness and last limb movement were longer in G-CO_2 relative to P-CO_2, differences were not observed for respiratory arrest. As expected, latency was increased by the use of P-Ar relative to P-CO_2 and G-CO_2. These results are consistent with previous work in our laboratory, in which G-CO_2 and a pre-filled 50:50 CO_2:Ar gas mixture were associated with 30 and 75% increased latencies to respiratory arrest when compared to P-CO_2 (Sadler *et al* 2014). Two DP pigs in the P-Ar treatment seemed to achieve respiratory arrest for more than 1 min and displayed no signs of sensibility during checks; however, these pigs recovered a regular gasping response. This anomaly highlights potential difficulty and unpredictability of Ar and warrants further exploration. As such, we would advise structuring guidelines for gas euthanasia around the latency to cardiac arrest (in this study: CO_2 ~15 min; Ar unknown since 60% of pigs reached the censored value). Cardiac arrest was the last detectable point in our study and a clear indicator of death, representing an appropriate and safe point to stop monitoring the euthanasia process in practice. Differences were not observed between gas treatments for cardiac arrest. This conflicts with Sutherland's (2011) findings, in which Ar prolonged the process compared to 100% CO_2. The absence of difference by gas type in the current study was surprising given that differences were observed between the gas treatments for all other measures of efficacy, but may be an artefact of our methods, including censoring of the pigs displaying prolonged responses as well as removing and returning pigs from the box to test insensibility.

Welfare implications — disease status

In this study, we separated the euthanasia process into two phases, conscious and unconscious. There is a transition phase prior to loss of consciousness during which a number of behaviours are typically observed, including open-mouth breathing, ataxia and righting response (Table 4; Forslid 1987; Raj & Gregory 1996; Sutherland 2011; Sadler *et al* 2014). The level of awareness, hence capacity of animals to suffer, during this transition is unclear, and we chose a conservative estimate by including all measures up to the point of loss of consciousness to ensure appropriate pig welfare. Behaviours chosen for welfare assessment included those associated with physiological distress, such as open-mouth breathing (Forslid 1987; Martoft *et al* 2002; Mota-Rojas *et al* 2012), or psychological distress, such as escape attempts (Blackshaw *et al* 1988; Velarde *et al* 2007) and righting response (Grandin 1998; Kohler *et al* 1999; National Pork Board 2009; AVMA 2013). When CO_2 was utilised at either flow rate, disease status did not affect any welfare parameters measured. However, in P-Ar, differences were observed in duration of open-mouth breathing, duration of ataxia and righting duration and intensity (number of efforts per pig).

Open-mouth breathing is a physiological reaction associated with dyspnea, and has been identified as an indicator of compromised welfare in the pig (Velarde *et al* 2007; Burki & Lee 2010). In P-Ar, duration of open-mouth breathing was approximately 3× greater for DP relative to OT. Durations of open-mouth breathing in P-CO_2 and G-CO_2 were similar to those reported previously in our lab (12 [± 2] and 24 [± 2] s for P and G, respectively; Sadler *et al* 2014). Ataxia and righting response durations and intensity (number of efforts per pig) were lower in the DP relative to the OT pigs, with duration of ataxia approximately 5× greater in OT. The duration of the righting response was decreased by half in the DP pigs relative to the

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OT pigs. Ataxia is likely an indicator of impaired function of the cerebellum (Guyton & Hall 2010); however, it is unclear how this correlates to impaired cortical function. If ataxia indicates that the pig is aware of its surroundings, but is unable to react in a co-ordinated manner, this could be considered distressing to the pig. In this study, we defined ataxia as a potential stressor for the pig, and hence, a shorter duration of this behaviour would correlate with improved welfare. The lack of a righting response has been cited as a critical indicator that a pig is successfully rendered unconscious prior to slaughter (Sandström 2009; Grandin 2010) and is cited as an indicator of unconsciousness (Anil 1991; National Pork Board 2009). The righting response requires co-ordinated brain activity (Deliagina *et al* 2008), and is an indicator of brain function. Since CO_2 and Ar are both heavier than air, it is possible that some of the righting responses observed reflect the animal's attempt to physically avoid the gas, as opposed to a reflexive behaviour. Hence, duration and intensity (frequency) of the righting response were used as indicators of distress in this study.

Animal welfare — gas treatment

Comparing gas treatments, differences were not observed in measured parameters of welfare between P-CO_2 and G-CO_2. P-Ar pigs had decreased welfare relative to P-CO_2 and G-CO_2, as measured by increased duration of open-mouth breathing, increased duration of ataxia, increased duration and intensity of righting response, and increased escape attempts. The observation of open-mouth breathing would suggest that peripheral chemoreceptors are activated prior to loss of consciousness. This is expected, since these peripheral chemoreceptors detect low O_2 and stimulate increased respiration in an effort to prevent loss of consciousness. Guyton and Hall (2010) report using the human as a model, a five-fold increase in respiration with the activation of the peripheral chemoreceptor while still conscious.

The results of the current study are consistent with results of a previous study from our laboratory using a similar protocol (G-Ar) and age of pig (Sadler 2013); relative to CO_2, Ar produced greater behavioural and physiological responses associated with reduced pig welfare during induction. Our findings indicating decreased welfare with Ar relative to CO_2 are also similar to those found for rats (*Rattus norvegicus*) by Sharp *et al* (2006). When CO_2 (10% BVE min^{-1}) vs Ar (50% BVE min^{-1}) was applied to modify the atmosphere to a concentration that would produce biologic effects in rats, convulsions and gasping were more frequently observed in Ar, whereas rats exposed to CO_2 showed no adverse reactions (Sharp *et al* 2006). Rats were not taken to loss of posture, and it is important to note that CO_2 aversion was observed in other rodent studies in which unconsciousness was induced (Hawkins *et al* 2006; Niel *et al* 2008). In humans, exposure to CO_2 has been associated with pain and coughing (Guyton & Hall 2010). In our study, sneezing or coughing were not observed in any of the gas treatments, which may indicate irritant receptors in the airways are not activated in pigs of this age, or perhaps this effect is not conserved among mammalian species. Results from the current study are also in agreement with Rault and colleagues (2013), who also removed the 100% Ar treatment due to ethical concerns.

The decreased welfare with the use of argon observed in the current study was surprising and conflicts with conclusions of researchers when Ar was applied to market-weight pigs (Raj & Gregory 1996) and with recommendations from EFSA (2004). Sutherland (2011) found that pigs exposed to Ar displayed an increase in the number and durations of vocalisations compared to pigs exposed to CO_2, suggesting greater distress. However, in the same study, decreased number and duration of escape attempts were performed by pigs exposed to Ar compared to CO_2, suggesting that pigs found CO_2 more aversive.

Efficacy — low weight

In general, weight did not have an effect on measures of efficacy, yet pigs weighing < 0.8 kg showed decreased latencies to measures of efficacy (respiratory arrest and cardiac arrest). This would support previous findings for pigs weighing < 0.8 kg as reported in Straw *et al* (1999), and indicates physiological differences that render them more susceptible to euthanasia. However, it is important to note differences described by Straw *et al* (1999) were in relation to birth weights, which is unknown in our pigs; the low weight here could represent pigs that had become severely emaciated.

Animal welfare implications

When utilising CO_2 as a euthanising agent for suckling pigs, at prefill or slow flow rates, depression status of the pig did not affect welfare or efficacy of the procedure. Conversely, depressed pigs responded differently and less predictably to Ar than pigs euthanised for other reasons. When utilised as a euthanising agent for suckling pigs, Ar reduced efficacy and welfare compared to CO_2 and should not be considered for use in gas euthanasia for this age of pig. These concerns are especially relevant in pigs with highly compromised health status.

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