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The effect of age and method of gas delivery on carbon dioxide euthanasia of pigs

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Abstract

*The objectives of this research were to evaluate the effect of age and delivering method during carbon dioxide (CO_2) euthanasia on the welfare of pigs (*Sus scrofa*). In Experiment 1, pigs aged 1, 2, 3, 4, 5 and 6 weeks (GRAD; n = 5 pigs per age) were placed in a chamber gradually filled with CO_2 released at a flow rate of 20% chamber volume per min. In Experiment 2, three week old pigs were placed in a chamber pre-filled with 100% CO_2 (PRE; n = 5). In both experiments, plasma cortisol concentrations were measured before and after exposure to CO_2. Behaviours indicative of stress and insensibility were recorded continuously during gas exposure; panting, open-mouth breathing, righting response, escape attempts, loss of posture, muscular excitation and respiratory arrest. Cortisol concentrations were elevated in pigs after CO_2 euthanasia, regardless of age or gas delivery method. The behavioural response to CO_2 was not affected by pig age. Latency to display panting, righting response, escape attempts, loss of posture, muscular excitation and respiratory arrest were shorter for PRE than GRAD pigs, but, duration of escape attempts and the cortisol response to euthanasia were similar between PRE and GRAD pigs. However, pigs placed in a chamber pre-filled with CO_2 spent a greater proportion of time prior to loss of posture displaying escape attempts. Regardless of age or induction method, exposure to CO_2 causes behavioural changes indicative of stress prior to loss of consciousness, therefore there is a need to continue to evaluate alternative methods of euthanasia.*

Keywords: *age, animal welfare, behaviour, carbon dioxide, euthanasia, pigs*

Introduction

There are times on a swine farm when pigs (*Sus scrofa*) become ill or injured, and the animal care person must decide if euthanasia is necessary and, if so, what method of euthanasia is most humane for the pig, while also considering worker stress. The American Veterinary Medical Association guidelines for the euthanasia of animals (AVMA 2013) suggests several methods of euthanasia that are acceptable for pigs less than 32 kg (nursery and suckling pigs). These include carbon dioxide gas (CO_2), intravenous administration of barbiturates and blunt-force trauma performed using a non-penetrating captive bolt or manually (manual blunt-force trauma is only acceptable for suckling pigs). Euthanasia of pigs using an injectable agent is not practical on-farm as barbiturates require special handling and licensing. Manually applied blunt-force trauma can be difficult to apply consistently and may be aesthetically unpleasant for operators to perform. In contrast, mechanically applied blunt-force trauma performed using a purpose-built, non-penetrating captive bolt can deliver an appropriate and uniform amount of force resulting in more consistent structural damage to the brain (Casey-Trott *et al* 2013, 2014), however, not all farms have access to these tools. In recent years, the US swine industry has encouraged the evaluation of alternative methods of euthanasia for pigs and particularly inhalant agents (Sadler *et al* 2014a).

Carbon dioxide causes death in animals by inducing acidosis and inhibition of neurons that leads to loss of consciousness, insensibility and finally death (European Food Safety Authority [EFSA] 2004). The advantages of CO_2 gas as a method of euthanasia are that it is relatively inexpensive, non-flammable and non-explosive. In addition, it is commonly used to stun pigs at slaughter in several countries. However, exposure to CO_2 can cause the formation of carbonic acid on respiratory and ocular membranes and the sensation of breathlessness and fear in animals (AVMA 2013). Due to the potential negative welfare implications of CO_2 there is a need to further evaluate this method of euthanasia.

There are two common methods of delivery when administering CO_2 to euthanase animals; pre-fill and gradual fill. The pre-fill procedure involves placing an animal in a closed chamber containing high concentrations of CO_2, typically greater than 70% (Hewett 1993; Coenen *et al* 1995; Smith & Harrap 1997). The gradual fill procedure involves placing an animal in a chamber containing atmospheric air only and gradually introducing CO_2 at a fixed-flow rate. Variable flow rates have been studied in the literature, ranging from 3 to 27% chamber volume per min in rats (Hewett 1993; Niel & Weary 2006; Niel *et al* 2008) and 20 to 50% chamber volume per min in pigs (Sadler *et al* 2014a). In rodents, there is evidence to suggest that animals exposed gradually to CO_2 at flow rates ranging from 3 to 27% experience aversion (Niel *et al* 2008) and when exposed to a chamber pre-filled with CO_2 at concentrations above 50% they experience pain prior to loss of consciousness (Hawkins *et al* 2006). However, the Newcastle Census Meeting (Hawkins *et al* 2006) concluded that exposing rodents to CO_2 using a flow rate of 20% appeared to produce loss of consciousness without evidence of pain. In pigs, Sadler *et al* (2014a) found that animals placed in a chamber pre-filled with 100% CO_2 displayed shorter durations of open-mouth breathing and ataxia, and a shorter latency to loss of posture, which is considered an indicator of the onset of loss of consciousness, compared to pigs exposed to CO_2 at a flow rate of 20% chamber volume per min. However, little is still known regarding the effects that these two gas-delivery methods have on the stress response in pigs.

Research in mice (Pritchett *et al* 2005) and rats (Pritchett-Corning 2009) suggests that neonates have a greater resistance to hypoxia and hypercarbia and hence higher CO_2 concentrations and/or extended exposure times may be necessary to ensure unconsciousness and death in very young animals. Conversely, Sadler *et al* (2014a) found that loss of posture occurred quicker in pigs less than two days of age compared to weaned pigs. However, little is known regarding the effect of CO_2 exposure on suckling and weaned pigs. Therefore, the objectives of this research were to evaluate the effect of delivery method and age during carbon dioxide euthanasia on the welfare of pigs.

Materials and methods

This study was conducted in April 2009 (northern hemisphere spring) at the Texas Tech University research swine farm, New Deal, Texas, USA. Pigs used in this study were PIC USA genetics (PIC North America, Hendersonville, TN, USA) using the Camborough-22 sow line. In this study, all pigs were healthy and viable as we did not want to confound health status, such as respiratory disease, with the effects of CO_2 euthanasia. All animal procedures involving animals were approved by the Texas Tech University Animal Care and Use Committee (#08046-12).

Euthanasia equipment

The clear plastic euthanasia chamber (76.6 × 50.0 × 42.6 cm; length × width × height) facilitated easy observation and recording of pig behaviour. The chamber had an inlet valve located on the right-hand side, 10 cm above the chamber floor, with a hose attached through which the CO_2 entered. An outlet valve was located on the opposite side of the chamber which had a tube attached to allow excess CO_2 to be ventilated outside the building. The CO_2 gas was industrial grade (99% pure). A non-slip surface was placed on the bottom of the chamber to prevent pigs from slipping.

The concentration of the CO_2 in the chamber was measured during euthanasia to calculate the 'wash-in and wash-out' effect of the CO_2. Carbon dioxide in the chamber was measured using a Pac III Personal Gas Monitor (Draeger Safety Inc, Gas Detections Systems, Sugarland, TX, USA).

Experiment 1

Female pigs from five different age classes were used in this study (n = 5 pigs per age class): 1 week (3.0 [± 0.13] kg); 2 weeks (3.7 [± 0.80] kg); 3 weeks (5.2 [± 0.72] kg); 4 weeks (7.1 [± 1.09] kg); 5 weeks (9.0 [± 1.44] kg); and 6 weeks (11.0 [± 0.42] kg). From birth until weaning, at approximately 28 days of age, pigs were housed in standard farrowing stalls (2.03 × 0.61 m; length × width) with the sow. Within the first three days after farrowing, piglets were routinely tail-docked, ear-notched (for identification) and given an iron injection (100 mg). At weaning, pigs were moved into the nursery and housed with unfamiliar conspecifics in groups of four or nine. Pens measured 3.6 × 2.1 m (length × width) and had woven wire flooring.

On the day of euthanasia, pigs were removed from their sow or the nursery pen, weighed and placed in a cart with sawdust bedding. They were then transported to an adjoining room which contained the euthanasia chamber and each pig placed in the chamber containing atmospheric air only and left in the chamber for 5 min prior to the gas being turned on in order to get baseline recordings of pig behaviour. The carbon dioxide was then released at a flow rate of 20% chamber volume per min. The flow rate was based on previous studies showing it to cause lower levels of distress in rodents (Hawkins *et al* 2006). Furthermore, the AVMA Euthanasia Guidelines (2013) recommend displacement with carbon dioxide of 10 to 30% of the chamber volume per min as an optimum flow rate for euthanasia.

Behaviour was recorded continuously at 30 frames per s using camcorders (Sony Handycam® Camcorder, DCR-SR85, Sony, Tokyo, Japan). The camcorder was placed on a tripod and positioned to view the side of the chamber. Behaviours measured included panting, open-mouth breathing, righting response, escape attempts, loss of posture, muscular excitation and respiratory arrest and are described in Table 1. One trained technician watched all videos.

Blood samples were collected immediately before the pig was placed in the chamber and once the pig was dead and its heart had stopped beating (absence of cardiac activity was confirmed by palpation). Pigs were placed in a v-trough in the supine position and 5 ml of blood collected into EDTA-coated vacutainers (BD, Franklin Drive, NJ, USA) via jugular venipuncture. Whole blood was centrifuged for 15 min at 1,000 g; plasma was aliquoted immediately and stored at –20°C for future analysis of cortisol concentrations using a commercially available kit with a sensitivity of

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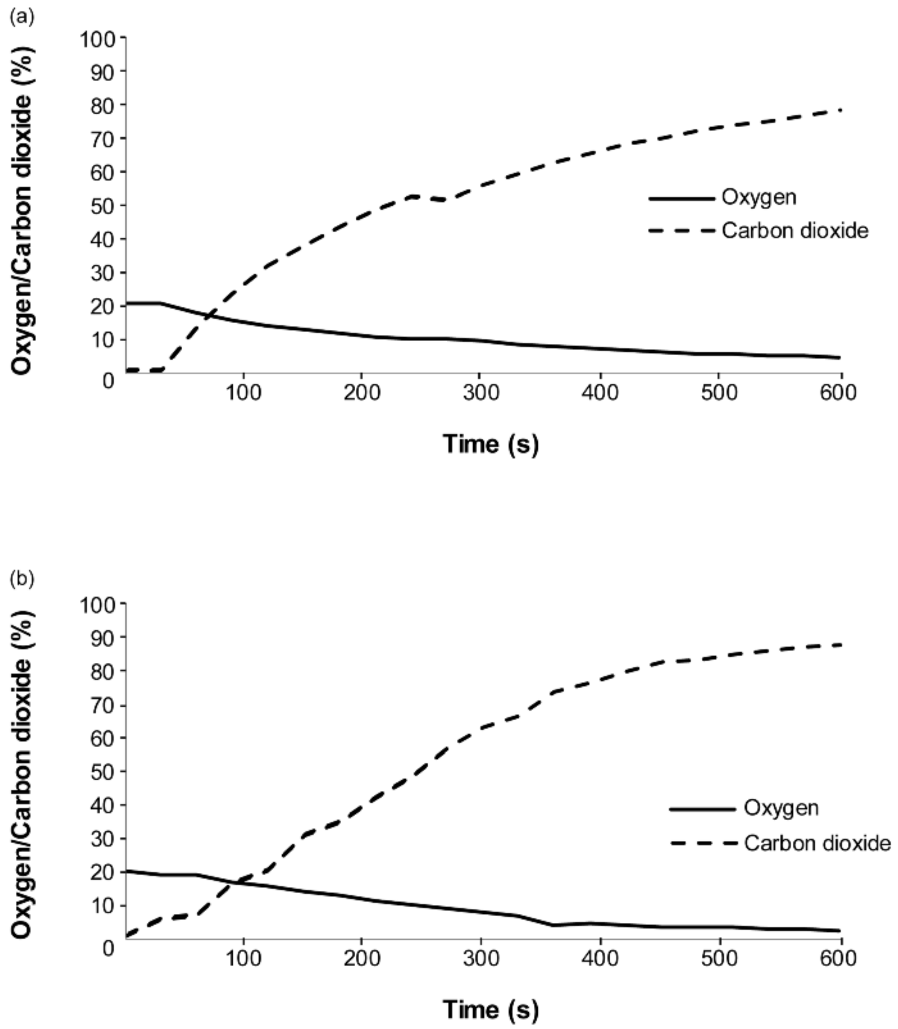
Table 1 Description of behaviours indicating distress and insensibility associated with exposure to carbon dioxide gas in neonatal pigs.

Behaviour	Description
Panting (latency)	Respiration rate approximately double the speed of normal respiration rate accompanied by flank movements from prominent inhalations and exhalations[1]
Open-mouth breathing (latency)	Pig's mouth is open while panting and there are distinct thoracic and flank movements associated with pronounced inhalations and exhalations[1,2,3]
Righting response (latency)	Pig is attempting to maintain either a standing or lying sternal posture, but appears unable to maintain this posture[2,3]
Escape attempt (latency and duration)	Pig's forelegs are raised on the sides and/or lid area of the chamber and the pig is pushing quickly and forcefully against the chamber using co-ordinated movements[2,3,4]
Loss of posture (latency)	Pig is lying recumbent on the floor of the chamber and making no attempt to right itself[2,4]
Muscular excitation (latency and duration)	Repeated muscular movements involving the whole body, including head movements upwards; these movements appear unco-ordinated. Severe excitation appears as clonic seizures[2,5,6]
Respiratory arrest (latency)	No thoracic movement visable[3]

Adapted from: [1] Velarde *et al* 2007; [2] Sadler *et al* 2014a; [3] Sadler *et al* 2014b; [4] Raj & Gregory 1996; [5] Rodríguez *et al* 2008; [6] Dodman 1977.

Figure 1



Oxygen and carbon dioxide percentages in euthanasia chamber over a 10-min period (a) when the chamber is empty and (b) when a pig is in the chamber. Time 0 is when the CO_2 gas cylinder was turned on with a flow rate of 20% container volume per min.

Table 2 Effect of age on latency and duration (least square means ± SEM) of neonatal pigs to display behavioural indicators of distress and insensibility.

Measures (s)	Age (weeks)						SEM	*P*-value
	1	2	3	4	5	6		
Latency								
Panting	17.0	21.3	26.0	31.7	27.2	25.3	5.2	0.444
Open-mouth breathing	46.9	64.3	62.1	79.9	73.9	86.9	9.2	0.068
Righting response	60.4	54.3	66.3	94.1	80.0	92.5	10.7	0.075
Escape attempt	111.3	85.3	95.3	121.7	109.1	105.0	9.2	0.115
Loss of posture	133.1	133.4	147.3	176.1	209.8	155.2	31.8	0.479
Muscular excitation	211.8	176.3	177.0	233.1	179.7	164.1	33.1	0.677
Respiratory arrest	408.4	361.5	482.5	433.0	377.1	416.0	34.0	0.169
Duration								
Escape attempt	8.7	24.3	16.5	13.5	27.5	30.9	8.5	0.419
Muscle excitation	60.7	64.4	73.2	58.9	119.1	55.2	21.0	0.303

0.56 ng ml^{-1} (Assay Designs, Ann Arbor, MI, USA). Samples were run in duplicate and the intra- and inter-assay coefficients of variation (CV) were 3.5 and 5%, respectively. Intra-sample CV was also calculated and samples with a CV greater than 12% were rerun.

Experiment 2

Five three week old female pigs were used in this study. Only one age group of pigs was used in this study for ethical reasons as the results from the previous study found no effect of age on the behavioural and physiological response to euthanasia using CO_2. Pigs were reared in the same way as in Experiment 1. On the day of euthanasia, pigs were removed from the sow, weighed and placed in a cart with sawdust bedding. They were then transported to an adjoining room which contained the euthanasia chamber. The pig was placed in the chamber containing 100% CO_2. After the pig had been placed in the chamber, CO_2 continued to be released into the chamber to compensate for any gas that may have escaped while the chamber was open and to determine that CO_2 concentrations within the chamber were at least at 90%. The same behavioural and physiological measures were collected as in Experiment 1.

Statistical analysis

Behaviours were recorded as latency or durations. Data were tested for homogeneity of variance and normal distribution then subjected to analysis of variance using the mixed model procedure of SAS version 9.3 (SAS Inst Inc, Cary, NC, USA). For analysis of age effects, only data from pigs used in Experiment 1 were included and the fixed effect of age was included in the model. For analysis of the effect of gas delivery method, data from three week old pigs (Experiment 1) and data from pigs used in Experiment 2 were included and the fixed effect of delivery method was included in the model. The pig was the experimental unit. Least square means for each group and the corresponding standard errors (± SEM) are reported. Statistical significance was determined at $P \leq 0.05$ and $0.05 < P \leq 0.10$ were considered a tendency. The sample size per age group or delivery method was determined by a power analysis with 80% power and 5% significance level.

Results

Gas dynamics in chamber

Carbon dioxide was released at a flow rate of 20% chamber volume per min and the percentage of oxygen and CO_2 gas were measured in the euthanasia chamber when it was empty (Figure 1[a]) and when it contained a pig (Figure 1[b]). The empty chamber reached CO_2 concentrations of 50% within approximately 4 min after the cylinder was turned on and a maximum concentration of 78% after 10 min. Oxygen and CO_2 concentrations within the chamber were similar with a pig in the chamber as compared to the empty chamber, across time. The chamber containing a pig reached CO_2 concentrations of 50% within approximately 4.5 min, 80% within 7.5 min and a maximum concentration of 87% at 10 min. Oxygen concentrations decreased simultaneously as CO_2 concentrations increased.

Effect of age

Cortisol concentrations were elevated ($P < 0.001$) in pigs after death caused by exposure to CO_2 at a flow rate of 20% chamber volume per min compared with baseline values, regardless of age (Baseline: 16.8 [± 4.52] ng ml^{-1}; Post mortem: 110.3 [± 7.00] ng ml^{-1}).

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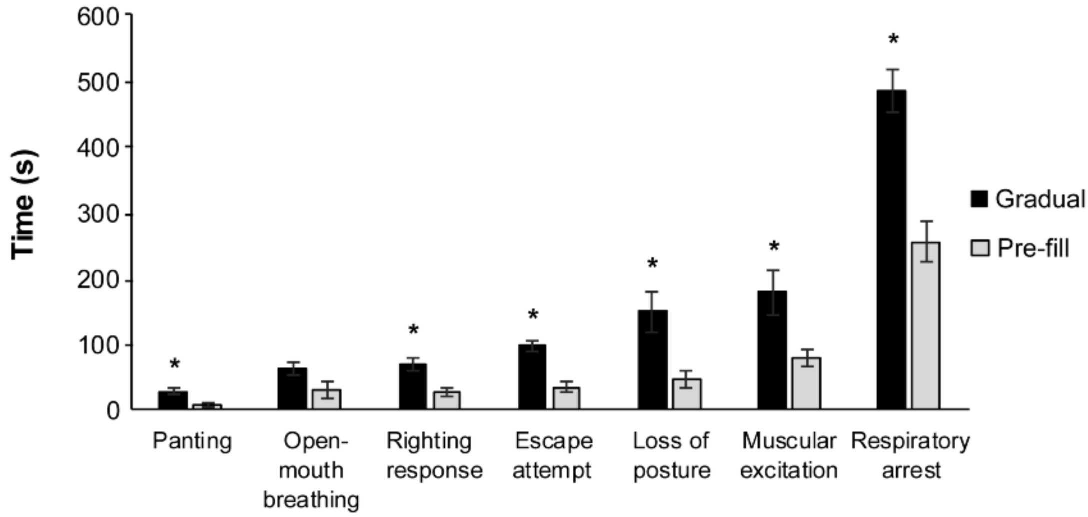
Figure 2



Effect of method of gas delivery on latency (least square means ± SEM) of neonatal pigs to display behavioural indicators of distress and insensibility. * Least square means differ at $P < 0.05$.

Age of pig did not affect ($P > 0.05$) latency to display panting, escape attempts, loss of posture, muscular excitation or respiratory arrest, or duration of escape attempts and muscular excitation (Table 2). However, latency to display open-mouth breathing and righting response tended to be affected by age (Table 2). The latency to display open-mouth breathing was shorter in 1 week than 4 ($P = 0.018$), 5 ($P = 0.049$) and 6 week ($P = 0.005$) old pigs. Also, 4 week old pigs took longer to display a righting response than 1 ($P = 0.017$) and 2 ($P = 0.015$) week old pigs.

Effect of gas delivery method

Cortisol concentrations were elevated ($P < 0.001$) in response to CO_2 euthanasia, regardless of CO_2 delivery method (Baseline: 11.5 [± 4.10] ng ml^{-1}; Post mortem: 97.5 [± 9.76] ng ml^{-1}). The cortisol response (post mortem – baseline concentrations) did not differ ($P = 0.109$) between methods (Gradual: 100.7 [± 11.48] ng ml^{-1}; Pre-fill: 71.4 [± 11.48] ng ml^{-1}).

Latency to display panting ($P = 0.004$), righting response ($P = 0.007$), escape attempts ($P < 0.001$), loss of posture ($P = 0.001$), muscular excitation ($P = 0.001$) and respiratory arrest ($P = 0.001$) were all shorter when pigs were placed in a chamber pre-filled with 100% CO_2 compared with pigs gradually exposed to CO_2 (Figure 2). Pigs placed in a pre-filled chamber tended ($P = 0.084$) to have a shorter latency to perform open-mouth breathing than pigs exposed to CO_2 gradually. However, duration of escape attempts (Gradual: 16.5 [± 4.9] s, Pre-fill: 10.8 [± 4.9] s; $P = 0.427$) and muscular excitation (Gradual: 73.2 [± 26.0] s, Pre-fill: 78.2 [± 26.0] s; $P = 0.896$) was similar between CO_2 delivery methods.

Discussion

Results from the present study indicate that the behavioural response of pigs gradually exposed to CO_2 at a flow rate of 20% chamber volume per min was similar among pigs between 1 and 6 weeks of age. Rodents (Pritchett *et al* 2005; Pritchett-Corning 2009) required higher concentrations and longer exposure times to CO_2 to cause unconsciousness and death when euthanased during the first days of life. Conversely, pigs less than two days of age displayed shorter latency to open-mouth breathe and lose posture than 3–4 week old pigs when exposed to CO_2 (Sadler *et al* 2014a). In the present study, latency and duration of behaviours and the cortisol response to gradual exposure to CO_2 was not affected by the age of the pig. Pigs are a precocious species and the respiratory part of the lung is more mature at birth than in rodents (Winkler & Cheville 1984), which may account for these species' differences in responsiveness to inhalation of CO_2.

In the present study, latency to open-mouth breathe and loss of posture was 47 and 133 s, respectively, in one week old pigs which is similar to the values reported by Sadler *et al* (2014a) for neonatal pigs (67 and 124 s, respectively). Additionally, latency to open-mouth breathe and loss of posture was 62 and 147 s, respectively, in three week old pigs in the present study, also similar to the values reported by Sadler *et al* (2014a) for weaned pigs (87 and 143 s, respectively). In the present study, latency to open-mouth breathe and loss of posture was numerically shorter in older animals, however the small sample size may account for lack of statistical differences. Overall, pigs appear to behave similarly in response to gradual exposure to CO_2 regardless of age, however, pigs may experience a lower degree of stress (or experience stress for a shorter duration) in response to CO_2 during the first week of life, however more research is needed to understand the mechanisms involved in this response.

The latency of pigs to perform all behaviours was shorter when they were placed in a pre-filled chamber compared to being gradually exposed to CO_2 in the present study. Sadler *et al* (2014a) also found that the latency to display open-mouth breathing and loss of posture was shorter for pigs placed in a chamber pre-filled with 100% CO_2. Moreover, Sadler *et al* (2014a) found that as flow rate increased latency to display these behaviours decreased. Loss of posture is an indicator of the onset of unconsciousness (Raj & Gregory 1996; Velarde *et al* 2007). In the present study, pigs placed in a pre-filled chamber also displayed loss of posture 70% quicker than pigs gradually exposed to CO_2. However, pigs exposed to a chamber pre-filled with CO_2 performed escape attempts for a similar duration as pigs gradually exposed to CO_2. Moreover, when the percentage of time pigs spent performing escape attempts was calculated in relation to the time prior to loss of posture, then pigs placed in a pre-filled chamber spent 24% of the time prior to loss of posture performing escape attempts compared with only 11% for gradually exposed pigs, which may suggest that placing pigs in a chamber pre-filled with 100% CO_2 causes a more aversive response, albeit for a shorter time. The performance of escape attempts is often associated with psychological distress in animals (Forkman *et al* 2007; Velarde *et al* 2007), therefore if loss of posture corresponds to loss of consciousness, pigs exposed to a chamber pre-filled with CO_2 are likely to have experienced a similar or greater degree of stress compared with pigs euthanased using the gradual-fill method (at a flow rate of 20%). However, if loss of posture does not signify loss of conscious, then these animals would definitely experience more distress.

Hawkins *et al* (2006) states that as a general rule, "a gentle death that takes longer is preferable to a rapid, but more distressing death". Moreover, they concluded that using a flow rate of 20% was a preferable method when using CO_2 to euthanase rodents, which is the same flow rate used in the present study. Sadler *et al* (2014a) found that pigs placed in a chamber pre-filled with CO_2 spent less time licking and chewing, open-mouth breathing, and ataxic than pigs exposed to CO_2 at a 20% flow rate. In the present study, duration of escape attempts and the cortisol response to euthanasia was similar among pigs placed in a pre-filled chamber and those gradually exposed to CO_2, but pigs placed in a pre-filled chamber spent a greater proportion of time prior to loss of posture displaying escape attempts. Therefore, the pre-fill method of CO_2 euthanasia is likely to cause similar if not a greater degree of stress to pigs than the gradual-fill method. Regardless, both the pre- and gradual-fill methods used in this study caused pigs to display behavioural signs of stress. Exposing animals to hypercapnic gases, such as CO_2, is likely to cause the sensation of breathlessness and thereby a negative affective state in animals (Beausoleil & Mellor 2015). Therefore, there is a need to evaluate alternative methods of euthanasia that cause less stress. Alternative gases, such as nitrous oxide (Rault *et al* 2013) or two-step methods that anaesthetise the animal prior to death (Rault *et al* 2015) show promise, but currently have their limitations or are not practical to implement on-farm. Alternatively, mechanically controlled blunt-force trauma performed using purpose-built devices have been shown to cause immediate loss of sensibility followed by death in pigs up to 9 kg (Casey-Trott *et al* 2013, 2014).

Animal welfare implications and conclusion

This study demonstrated that between a range of 1 to 6 weeks, age of pig did not affect the behavioural response to CO_2 euthanasia when CO_2 was administrated gradually at a flow rate of 20% chamber volume per min. Placing pigs in a chamber pre-filled with CO_2 reduced the latency to loss of posture compared with pigs gradually exposed to CO_2, but this method may be more aversive and both methods of CO_2 exposure cause stress. Consequently, exposure to CO_2 regardless of method of induction or age causes an increase in cortisol concentrations and behavioural changes indicative of stress prior to loss of consciousness. Therefore, there is a need to continue to evaluate alternative methods of euthanasia that cause immediate and sustained insensibility and death and which can also be practically applied on-farm.

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