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# Evaluation and application of humane hypoxia euthanasia for nursery pigs

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## Abstract

Fifty-eight nursery pigs ( $5.6 \pm 1.3$  kg) were utilized to compare physiological, behavioral, and neurophysiological parameters of hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanasia. This experiment was conducted as a completely randomized design using a  $2 \times 2$  factorial arrangement of treatments. Factors included euthanasia method: A) hypobaric hypoxia (approximate ascension of 36.9 m/sec) or B) CO<sub>2</sub> gas (induction of approximately 20% of the chamber volume/minute) and 2) health status: A) healthy or B) moribund. Health classification was determined by a veterinarian. Two pigs at a time were euthanized using a 1 m<sup>3</sup> chamber for each method by health status treatment ( $n = 8$ , CO<sub>2</sub>-moribund;  $n = 8$ , CO<sub>2</sub>-healthy;  $n = 8$ , HH-moribund;  $n = 5$ , HH-healthy). Jugular blood samples were obtained from each pig 24 hours prior to euthanasia. Animals were fitted with ECG and EEG monitoring devices, placed in the chamber and kept in the chamber until death was confirmed via ECG and EEG. In addition to ECG and EEG measurements, behavioral parameters were measured and necropsies were performed. Post-euthanasia, a blood sample was obtained from each pig. The average treatment times were HH,  $27.4 \pm 6.7$  minutes and CO<sub>2</sub>,  $13.8 \pm 5.1$  minutes. The EEG data indicated that pigs euthanized via CO<sub>2</sub> reached the point of a complete isoelectric state faster than pigs euthanized via HH ( $P = 0.009$ ; HH:  $13.4 \pm 5.6$  min; CO<sub>2</sub>:  $7.8 \pm 8.7$ ). When evaluating the average power of EEG waves in pigs euthanized via hypobaric hypoxia, there was no interaction between health status and time ( $P = 0.84$ ). Conversely, there was a time by health status interaction ( $P = 0.005$ ) when evaluating the dominant frequency of EEG waves. At approximately 10,600 m, healthy pigs exhibited higher dominant frequency values than moribund pigs ( $P = 0.0002$ ). When evaluating the average power of EEG waves in pigs euthanized via CO<sub>2</sub>, there was a time effect ( $P < 0.0001$ ). As time progressed, the average power of EEG waves decreased. Similar to dominant frequency values in HH pigs, there was a time by health interaction ( $P = 0.0007$ ). Healthy pigs exhibited higher ( $P < 0.0001$ ) dominant frequency values than moribund pigs after 9-10 minutes of CO<sub>2</sub> induction. Pulmonary lesions were present in 20.7% of hypobaric hypoxia euthanized pigs and only

1.7% of pigs via CO<sub>2</sub> euthanasia ( $P < 0.0001$ ). Moribund pigs euthanized via CO<sub>2</sub> tended ( $P = 0.08$ ) to have less bouts of paddling than moribund pigs euthanized via HH. Additionally, healthy pigs euthanized via CO<sub>2</sub> tended ( $P = 0.09$ ) to exhibit more paddling bouts than moribund pigs euthanized via CO<sub>2</sub>. Pigs euthanized via CO<sub>2</sub> gasped more ( $P = 0.01$ ) than pigs euthanized via hypobaric hypoxia. Pigs euthanized via HH fell down more than pigs euthanized via CO<sub>2</sub> ( $P = 0.01$ ). Blood samples were analyzed for lactate ([LAC]), glucose, ionized calcium (iCa), potassium, hemoglobin, and sodium concentrations, percent hematocrit, pH and partial pressures of oxygen ( $pO_2$ ) and CO<sub>2</sub> ( $pCO_2$ ). A health status effect ( $P \leq 0.004$ ) was observed for hematocrit, hemoglobin, iCa, [LAC] and sodium ( $P \leq 0.004$ ). Moribund pigs had higher ( $P \leq 0.05$ ) hemoglobin and sodium concentrations, and percent hematocrit than healthy pigs. There was an interaction ( $P \leq 0.03$ ) between sample time (pre-euthanasia vs. post-euthanasia) and treatment (HH vs. CO<sub>2</sub>) for [LAC], glucose, hematocrit, hemoglobin, iCa, potassium, sodium and  $pCO_2$ . Post-euthanasia glucose concentrations were higher ( $P < 0.0001$ ) in pigs euthanized via CO<sub>2</sub> compared to HH. However, post-euthanasia percent hematocrit, hemoglobin concentration, [LAC], and pH were higher ( $P = 0.03$ ) in HH compared to CO<sub>2</sub> euthanized pigs. There were no differences for epinephrine or norepinephrine between euthanasia method ( $P = 0.21$ ) or health status ( $P = 0.62$ ) of piglets. There was a sample by health interaction ( $P = 0.04$ ) for cortisol. Moribund pigs exhibited higher post-euthanasia cortisol values than healthy pigs ( $P = 0.001$ ).

## Introduction

Hypoxia experiments have been performed extensively and, as a result, the effects are well documented and understood. Though hypobaric hypoxia experiments have been performed as well, they generally do not focus on the distress and discomfort of the animals themselves. Many experiments involving hypobaric hypoxia are performed to test and isolate effects of high altitude environments on animals acclimated to relatively low altitudes (Wittner, 2005). The most common symptoms of hypobaric hypoxic effects include but are not limited to: euphoria,

empowerment, intoxication, friendliness, sleepiness, and dizziness (Smith, 1965; Booth, 1978; Shukitt-Hale, 1997). As duration of high altitude exposure increases, different feelings such as depression, irritability, and quarrelsomeness are experienced (Shukitt-Hale, 1997). Unconsciousness and death caused by low rapid and gradual decompression is the result of hypobaric hypoxia. Hypobaria and hypoxia describe two separate conditions which can exist independently or simultaneously. Hypoxia is experienced when one's blood oxygenation is at an inadequate level (Dictionary, 2007). Causing hypoxia independent of hypobaria can be accomplished by inhaling gases which contain lower partial pressures of oxygen. Any euthanasia method which utilizes an inhalant agent relies on lethal levels of hypoxia to induce death, such as CO<sub>2</sub> gas. Hypobaria is described as a decrease in ambient pressure on a body (Medical Dictionary, 2007). Similar to hypoxia, hypobaria can be experienced independently though it is less common. Hypobaric conditions exist at high elevations, but an oxygen supply is needed to isolate hypobaria. Hypobaric hypoxia can occur at a high altitude without an additional oxygen supply. Pilots and mountain climbers often use oxygen supplies to avoid hypoxic effects of high elevation environments.

Current engineering control technology provides an opportunity to maximize the early symptoms of hypobaric hypoxia. In the past, experiments using decompression to prevent blood oxygenation in animals to achieve death showed indications of severe pain and distress. Therefore, processes utilizing decompression were labeled inhumane and are currently unacceptable to use on any animal. Due to lack of technology and adequate understanding, the decompression experiments failed to allow precise control of airflow and chamber pressure. As a result, the vacuum systems were decompressed at an uncontrolled, maximum rate.

Past literature and extensive human experience suggest that slower decompression rates may cause less distress and pain in animals. Therefore, a prototype (MacGregor, 2008) was constructed to isolate the symptoms of hypobaric hypoxia induced by gradual decompression. The prototype was successful in accomplishing the task of creating a reliable and repeatable way to induce hypobaric hypoxia in mice. The system used closed loop control to automatically decompress a vacuum chamber at a consistent and predetermined rate. The automated system was found to be necessary when performing gradual decompression because the required flow control is too delicate for a human to manually perform. Many experiments have been completed utilizing this prototype with no failures and consistent results. The observational criteria noted during decompression were compared to that of current humane euthanasia methods and were used to evaluate

the relative amount of distress and pain experienced by the mice. After preliminary and full system tests, it was found that gradual decompression can cause euthanasia in mice. Gradual rates of ascent below 45.7 m/sec were used to achieve a simulated elevation of 15,000 m with a pause of three minutes at 10,000 m. Based on these results hypobaric hypoxia seems to be a humane alternative to current mouse euthanasia methods.

Based on previous experimentation, it is plausible that if the simulated altitude an animal experiences is arrived at gradually enough to not cause pain yet fast enough to avoid negative reactions or adaptation and to a high enough elevation to which the oxygen supply is inadequate and causes hypoxia, stress would be minimized during euthanasia. Therefore the objective of this experiment was to determine the behavioral, biochemical and physiological response to hypobaric hypoxia euthanasia in nursery pigs.

## Materials and methods

The Colorado State University and Kansas State University Institutional Animal Care and Use Committees approved protocols used in this experiment. Prior to the main experiment, two test experiments were conducted in order to determine the effectiveness of the modified hypobaric chamber and to determine the optimal ascension rate to euthanize pigs.

Fifty eight nursery weight pigs were utilized in this experiment; twenty-six pigs were euthanized via hypobaric hypoxia (approximately 36.9 m/sec) and thirty-two via CO<sub>2</sub> gas induction at 20% the volume of the chamber per minute. Pigs were categorized (by a veterinarian) into two health categories: healthy or moribund, and euthanized via CO<sub>2</sub> or hypobaric hypoxia two pigs at a time (experimental unit: n = 8, CO<sub>2</sub>-moribund; n = 8, CO<sub>2</sub>-healthy; n = 8, HH-moribund; n = 5, HH-healthy). The experiment was conducted at a commercial swine facility over three days. Approximately 24 hours prior to euthanasia, 10 ml of blood was obtained from each piglet via jugular venipuncture into two tubes: 1) 9 ml K<sub>3</sub>EDTA tube (#22-040-037 Fischer Scientific, Pittsburgh, PA) for analysis of cortisol, epinephrine and norepinephrine and 2) 6 ml lithium heparin tube (#02-687-97 Fischer Scientific, Pittsburgh, PA) for analysis of blood gases via an iSTAT Analyzer (CG8+ #600-9000-25, Abaxis, Union City, CA). At the time of blood sampling, pigs were weighed, ear tagged and marked with a livestock chalk marker.

The euthanasia chamber was 1 m<sup>3</sup> with solid sides and a plexiglass lid for behavioral observation. Within the chamber, there was a video camera and a sling similar to one developed by Panepinto (1983) that would accommodate one pig. Prior to euthanasia, a pig was placed in the sling and fitted with EEG electrodes (152.4 cm lead

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wire, Chalgren Enterprises, Inc. #112-812-60TP). Two electrodes were placed behind each ear, five were arranged on top of the piglet's head and two ECG leads were placed in the front and rear flank of the pig to measure heart rate. The lid was left open and the chamber left inactive for approximately 5 min after inserting the electrodes in order to obtain sufficient EEG baseline values for comparison to treatment values. At the conclusion of the baseline observation, a second pig was placed on the floor of the chamber, the lid was closed and the euthanasia treatment was applied.

During euthanasia, animal behaviors were recorded on video. Video was analyzed and the number of occurrences of certain behaviors in a 25 min period were recorded. The average treatment times were hypobaric hypoxia,  $27.4 \pm 6.7$  minutes and carbon dioxide,  $13.8 \pm 5.1$  minutes. Pigs on the floor of the chamber were monitored for paddling, gasping, convulsing and number of times the animal fell down. Pigs restrained in the Panepinto-like sling were monitored for the number of occurrences of gasping, struggling, convulsing and vocalization. Vocalizations were not recorded from pigs on the floor due to the lack of audio capabilities of the camera inside the chamber.

Death of the pig in the sling was confirmed via EEG and ECG by an electrophysiologist. Death of the pig on the floor was determined by visual observation of a cessation of movement and respiration for five minutes. Upon confirmation of death, the chamber was returned to ambient environmental conditions and pigs were removed from the chamber. An additional 10 ml blood sample was drawn post mortem directly from the heart prior to opening the body cavity for necropsy.

Piglets were necropsied after removal from the chamber. Piglets were classified into three categories: pigs with pre-existing lesions, pigs with significant pulmonary lesions, and pigs with no significant lesions. Definitions of categories are as follows:

**Pre-existing lesions:** Pre-existing lesions were defined as gross evidence of disease that was not related to euthanasia. These included numerous abscesses in the subcutis, mesentery, umbilicus, and liver. Also included in this category were arthritis, marked abdominal fluid, hydronephrosis, hydrocephalus, diarrhea, hepatic lipidosis, rhinitis, and serous atrophy of fat (indicating severe caloric deficit).

**Pigs with significant pulmonary lesions:** Lesions interpreted as significant and were interpreted as being related to the euthanasia process, localized in the lungs, but excluding cyanosis. Cyanosis appears as pale blue mucous membranes of the oral cavity, tongue and the skin of the nose and was excluded from this category because it is expected from either method of euthanasia. These

lesions included partial atelectasis or partially collapsed lungs (lungs were less inflated than a normally collapsed lung post-mortem), and severe pulmonary edema of the majority of the lungs, that resulted in the lungs sinking in water, or lungs that sank initially and rose slowly, indicating that there was less edema. Lesions that were excluded from this category included mild congestion and edema where the lungs rose quickly in water or did not sink at all. Also excluded from this category were mild non-specific lesions such as ascites or small amounts of clear fluid in the pleural space or pericardial sac, and all of the pre-existing lesions.

**No significant lesions:** The pigs in this category had no gross lesions related to a known disease process, or lesions associated with euthanasia, but included mild non-specific changes as described above, and included both sick and healthy pigs as well as pigs from both euthanasia methods. This category includes pigs from any clinical disease category (sick and healthy). These lesions included mild pulmonary congestion, pulmonary petechial, and small amounts of abdominal or pleural fluid.

Organs examined during necropsy included: brain, heart, lungs, trachea, oral cavity, integument, shoulder/hip/stifle joints, thoracic cavity, esophagus, diaphragm, liver, spleen, pancreas, stomach, umbilicus, urinary bladder, urachus, kidneys, ureters, stomach, small intestine, large intestine, cecum, mesentery, reproductive organs, and mesenteric lymph nodes. Piglet carcasses were disposed of by the owner of the commercial facility.

**Blood analysis:** Prior to being centrifuged, whole blood was analyzed for lactate concentration [LAC] with a hand-held lactate analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany) and for glucose, ionized calcium (iCa), potassium, hemoglobin, and sodium concentrations, hematocrit, pH and partial pressures of oxygen (pO<sub>2</sub>) and carbon dioxide (pCO<sub>2</sub>) using an iSTAT clinical analyzer and cartridge (CG8+ #600-9001-25, Abaxis, Union City, CA).

Blood samples were centrifuged on site at the commercial facility. Samples were centrifuged for 15 min at  $1000 \times g$  and stored on ice until transport to Kansas State University. Upon returning to Kansas State University samples were stored at  $-20^{\circ}\text{C}$ .

Plasma cortisol was analyzed using a COAT-A-COUNT Kit (#TKC01, Diagnostic Products Corporation, Los Angeles, CA) using a Packard Cobra Gamma Counter (PerkinElmer, Waltham, MA). Plasma epinephrine and norepinephrine were isolated using activated alumina and 0.1M HClO<sub>4</sub> and quantified in duplicate using HPLC as described by Holladay and Edens (1987). Each plasma sample received 250 ng of 3,4-dihydroxybenzylamine hydrobromide (DHBA) which served as the internal standard.

Catecholamine:DHBA peak height ratios for samples and standards were determined and sample catecholamine concentrations were calculated using the regression equation generated from each catecholamine standard. Recovery of the internal standard ranged from 83-87% and duplicate samples were averaged when coefficients of variation (CV) were less than or equal to 5%. Duplicate samples with CV greater than 5% were re-analyzed until variation was within the acceptable limits.

## Electroencephalogram

Electroencephalogram data was analyzed and summarized by an electrophysiologist. Average power of waves was measured at different points in each treatment (HH: baseline, 7010 m, 7900 m, 9700 m, 10600 m, 10 minutes after 7,010 m (elevations are approximate); CO<sub>2</sub>: baseline, a few seconds after initiation of CO<sub>2</sub> induction, 5 min, 7 min and 9-10 min). Measuring the average power is a way to observe whether the 'energy' of the brain is increasing or decreasing over time. Dominant frequency was measured at the same time points as average power. Dominant frequency is the frequency value that has the most power within the EEG wave.

Electroencephalogram waves were also evaluated in order to determine the amount of time each treatment took to elicit an isoelectric response from the piglet. When evaluating data from CO<sub>2</sub> euthanized pigs, time was measured from the start of treatment to the emergence of an isoelectric EEG accompanied with ECG artifacts and then from that point to a totally isoelectric EEG. For pigs euthanized via hypobaric hypoxia, time was measured from the point at which 7,010 m was reached to when an isoelectric EEG was seen accompanied by ECG artifacts. As with CO<sub>2</sub> pigs, time from the artifacts point to a totally isoelectric EEG was also measured. During euthanasia for several pigs, the EEG electrodes became dislodged from the pig due to excessive struggling by the pig. EEG data for 14 pigs is included in this analysis.

## Electrocardiogram

Heart rate data was collected from ECG recordings collected during treatment. Heart rate was measured during baseline measurements and then again at various points during treatment. Heart rate for pigs euthanized via HH was measured at an equivalent elevation of 7010 m and then for two consecutive minutes afterwards (one value per minute). Heart rate for pigs euthanized via CO<sub>2</sub> induction was measured at the start of induction and then for three consecutive minutes afterwards (one value per minute).

Statistical analyses was conducted using SAS 8.2 (SAS Institute Inc., Cary, North Carolina, USA). Blood data, EEG (time to isoelectric signal) and behavioral data were

analyzed using PROC MIXED with the fixed effects of euthanasia method, health status and sample time. ECG data, EEG average power and dominant frequency were analyzed separately for CO<sub>2</sub> and HH as time points were not comparable between methods. Day was used as a blocking parameter in the statistical analysis of blood parameters and no block interactions were observed so it was removed from the analysis. Necropsy data were analyzed using the PROC FREQ CHISQ function. The Kendwardroger approximation was used to calculate denominator degrees of freedom. Pig was a random effect in all analyses.

## Results

**Blood analysis:** A health status effect was observed for percent hematocrit, [hemoglobin], [iCa], [sodium], and [LAC] ( $P \leq 0.02$ ). Moribund pigs had higher hemoglobin and sodium concentrations, and percent hematocrit than healthy pigs. Results indicated an interaction ( $P \leq 0.03$ ) between sample time (pre-euthanasia vs. post-euthanasia) and treatment (HH vs. CO<sub>2</sub>) for [LAC], glucose, hematocrit, hemoglobin, iCa, potassium, sodium and  $p\text{CO}_2$ . Post-euthanasia glucose concentrations were higher ( $P < 0.0001$ ) in pigs euthanized via CO<sub>2</sub> compared to pigs euthanized via HH. However, post-euthanasia percent hematocrit, hemoglobin concentration, sodium, [LAC], and pH were higher ( $P \leq 0.03$ ) and  $p\text{CO}_2$ , iCa and potassium were lower ( $P < 0.02$ ) in pigs euthanized via HH compared to CO<sub>2</sub>. Higher concentrations of hemoglobin and hematocrit are associated with exposure to high altitudes. It is unclear whether these changes could have occurred during the short length of the euthanasia treatment. A higher blood pH in HH pigs may be an example of the animal trying to metabolically reach a more stable pH. Higher glucose and [LAC] concentrations may be attributed to differences in length of time the animals remained in the chamber thus altering concentrations of post-mortem metabolites. There were no differences for epinephrine or norepinephrine between euthanasia method ( $P < 0.21$ ) or health status ( $P = 0.62$ ) for piglets. There was a sample (pre- vs. post-euthanasia sample) effects for both epinephrine and norepinephrine ( $P < 0.0001$ ), post-euthanasia values being higher ( $P < 0.0001$ ) for both blood parameters.

Results for cortisol indicated a sample by health interaction ( $P = 0.05$ ). Moribund pigs exhibited higher ( $P = 0.001$ ) post-euthanasia cortisol concentrations than healthy pigs. These values indicate that moribund pigs may have been experiencing a greater amount of stress than healthy pigs. Furthermore, values may have differed because the blood could not be drawn immediately at time of death, i.e. cortisol concentrations may have increased after death but prior to blood sampling. Blood data values are reported in Table 1.

**Table 1:** Effects of euthanasia method and health classification on blood metabolites in nursery pigs.

	Pre-euthanasia				Post-euthanasia				P-Value	
	HH	CO <sub>2</sub>	Morib.	Healthy	HH	CO <sub>2</sub>	Morib.	Healthy	Method	Method × Sample
Lactate (mmol/L)	3.7 ± 0.47	3.7 ± 0.42	3.5 ± 0.43	3.9 ± 0.47	16.9 ± 0.47	12.6 ± 0.42	13.7 ± 0.43	15.8 ± 0.47	< 0.0001	0.61
Cortisol (ng/ml)	19.0 ± 13.58	19.5 ± 12.87	21.9 ± 12.96	16.6 ± 13.87	71.3 ± 13.10	46.6 ± 12.41	77.6 ± 12.59	40.4 ± 13.09	< 0.0001	0.37
Glucose (mg/dL)	86.0 ± 20.60	87.5 ± 18.07	78.3 ± 18.07	95.3 ± 20.60	184.7 ± 20.60	321.0 ± 18.3	243.4 ± 18.07	262.4 ± 20.85	< 0.0001	0.67
Hemoglobin (g/dL)	10.6 ± 0.38	11.0 ± 0.34	11.7 ± 0.34	9.9 ± 0.38	13.1 ± 0.38	11.4 ± 0.34	13.0 ± 0.34	11.4 ± 0.39	< 0.0001	0.88
Hematocrit (% PCV)	31.3 ± 1.17	32.3 ± 1.00	34.5 ± 1.03	29.1 ± 1.17	38.3 ± 1.17	33.4 ± 1.00	38.1 ± 1.03	33.7 ± 1.18	< 0.0001	0.83
iCa (mmol/L)	1.3 ± 0.02	1.3 ± 0.02	1.3 ± 0.02	1.4 ± 0.02	1.4 ± 0.02	1.5 ± 0.02	1.4 ± 0.02	1.5 ± 0.02	< 0.0001	0.55
Sodium (mmol/L)	135.9 ± 0.91	134.7 ± 0.80	133.7 ± 0.80	136.9 ± 0.91	138.7 ± 0.91	135.0 ± 0.80	135.1 ± 0.79	138.7 ± 0.91	0.007	0.15
Potassium (mmol/L)	5.6 ± 0.20	5.4 ± 0.18	5.6 ± 0.18	5.4 ± 0.20	7.3 ± 0.20	7.9 ± 0.18	7.3 ± 0.18	7.9 ± 0.20	< 0.0001	0.24
pCO <sub>2</sub> (mmHG)	44.8 ± 2.90	42.3 ± 2.54	41.1 ± 2.54	46.0 ± 2.90	112.3 ± 2.90	130.0 ± 2.58	120.3 ± 2.54	121.9 ± 2.94	< 0.0001	0.95
PO <sub>2</sub> (mmHG)	45.3 ± 3.44	45.3 ± 3.02	49.5 ± 3.02	41.1 ± 3.44	28.1 ± 3.44	27.5 ± 3.07	28.4 ± 3.02	27.2 ± 3.49	< 0.0001	0.93
pH	7.4 ± 0.02	7.4 ± 0.02	7.4 ± 0.02	7.4 ± 0.02	6.7 ± 0.02	6.6 ± 0.02	6.6 ± 0.02	6.7 ± 0.02	< 0.0001	0.40
Epinephrine (ng/ml)	0.2 ± 0.84	0.1 ± 0.72	0.1 ± 0.72	.01 ± 0.84	13.1 ± 0.84	13.9 ± 0.72	13.9 ± 0.72	13.1 ± 0.84	< 0.0001	0.09
Norepinephrine (ng/ml)	0.2 ± 0.75	0.2 ± 0.64	0.2 ± 0.64	0.2 ± 0.75	15.7 ± 0.75	17.6 ± 0.64	16.5 ± 0.64	16.8 ± 0.75	< 0.0001	0.44

**Necropsy data:** There was unequal distribution of pre-existing lesions between the moribund and healthy pigs (16 moribund/3 healthy). The most common lesion related to euthanasia regardless of method was cyanosis of the lips, nose, and tongue (40 pigs). Five pigs showed no signs of any significant lesions. The most common lesion related to euthanasia apart from cyanosis was pulmonary edema and congestion, and pulmonary atelectasis. One pig had diffuse subcutaneous emphysema as well as pulmonary congestion. Eleven pigs had non-specific lesions such as epicardial petechia, and mild peritoneal and pericardial fluid accumulation without fibrin. Four of the eleven had mild pulmonary congestion that was considered insignificant, and the remaining seven had significant pre-existing lesions. Significant pulmonary lesions related to the euthanasia occurred with higher frequency in the HH treated pigs. Post euthanasia lesions were present in 20.7% of HH euthanized pigs and only 1.7% of pigs via ( $P = 0.001$ ) CO<sub>2</sub> euthanasia ( $P < 0.0001$ ). There was no difference in occurrence of lesions between healthy and moribund pigs ( $P = 0.23$ ).

Many pigs had cyanosis resulting from lack of oxygen, which appeared as pale blue mucous membranes of the oral cavity, tongue, and the skin of the nose. Some pigs had small 3-5 mm diameter pustules in the epidermis with surrounding erythema and swelling. One pig euthanized via HH had diffuse emphysema of the subcutaneous tissues which appeared as small clear air bubbles in the fat and fascia of the subcutis. One pig had a small amount of mucoid exudate in the nasal passages. Lung lesions ranged from severe diffuse pulmonary edema to mild multifocal pleural petechia. Severe edema was characterized by dark red, rubbery, wet, heavy lungs which sank in water. Milder lesions looked similar but were restricted to the caudo-dorsal lobes or the cranial lobes, and sank initially in water but rose slowly, indicating less severe edema. Some pigs had lungs which were mildly collapsed beyond the normal post mortem collapse of lungs when the thoracic cavity is breached. This is an indication that the pressure in the pleural space was greater than the external atmospheric pressure, and was seen only in pigs that died from hypobaric hypoxia in the early stages of the experiment.

**Electroencephalogram data:** Results of EEG data, in terms of total treatment time observed until the appearance of an isoelectric EEG, demonstrated that time to onset of respiratory distress was not different between HH ( $8.9 \pm 3.8$  min) and CO<sub>2</sub> ( $5.1 \pm 9$  min) euthanized pigs ( $P = 0.80$ ) or healthy pigs ( $7.4 \pm 3.0$  min) and moribund pigs ( $6 \pm 3.1$  min;  $P = 0.42$ ). Additionally, there were no differences in the amount of time elapsed from the onset of respiratory distress to the point of an isoelectric EEG accompanied by an ECG signal. However, when evaluat-

ing the length of time elapsed from the appearance of an isoelectric EEG with an ECG signal to the point of a completely isoelectric EEG signal (with no ECG artifacts), there was a treatment effect ( $P = 0.009$ ); pigs euthanized via CO<sub>2</sub> reached the point of a completely isoelectric state faster than pigs euthanized via HH. The average treatment time for HH and CO<sub>2</sub> were  $27.4 \pm 6.7$  minutes and  $13.8 \pm 5.1$ , respectively. These results indicate that euthanasia via CO<sub>2</sub> is quicker than HH.

When evaluating the average power of EEG waves in pigs euthanized via HH, there was no effect of health status ( $P = 0.98$ ) or time ( $P = 0.15$ ). Conversely, there was a time by health status interaction ( $P = 0.005$ ) in the evaluation of dominant frequency of EEG waves. At approximately 10600 m, healthy pigs exhibited higher ( $P = 0.0002$ ) dominant frequency values than moribund pigs.

When evaluating the average power of EEG waves in pigs euthanized via CO<sub>2</sub>, there was a time effect ( $P < 0.0001$ ). As time progressed, the average power of EEG waves decreased. Similar to dominant frequency values in HH pigs, there was a time by health interaction ( $P = 0.0007$ ) in the dominant frequency of CO<sub>2</sub> euthanized pigs. Healthy pigs exhibited higher ( $P < 0.0001$ ) dominant frequency values than moribund pigs after 9-10 minutes of CO<sub>2</sub> induction.

**Electrocardiogram data:** There were no heart rate differences from health status ( $P = 0.10$ ) or over time ( $P = 0.32$ ) in pigs euthanized via HH. Similarly, there was no health status effect for CO<sub>2</sub> euthanized pigs ( $P = 0.93$ ). However, there was a difference ( $P = 0.01$ ) observed over time. As the length of time exposed to CO<sub>2</sub> increased, heart rate decreased.

**Behavioral data:** When evaluating behavioral data from pigs on the floor of the chamber, there were no differences for convulsing bouts between either euthanasia method ( $P = 0.26$ ) or health status ( $P = 0.77$ ). A method by health interaction was observed for paddling ( $P = 0.04$ ). Moribund pigs euthanized via CO<sub>2</sub> tended ( $P = 0.08$ ) to have less bouts of paddling than moribund pigs euthanized via HH. Additionally, healthy pigs euthanized via CO<sub>2</sub> tended ( $P = 0.09$ ) to exhibit more paddling bouts than moribund pigs euthanized via CO<sub>2</sub>. There was a significant ( $P = 0.01$ ) method effect for gasping. Pigs euthanized via CO<sub>2</sub> gasped more than pigs euthanized via HH ( $P = 0.01$ ). Additionally, pigs euthanized via HH fell down more ( $P = 0.01$ ) frequently than pigs euthanized via CO<sub>2</sub>.

There were no differences between euthanasia methods or health status for any other observed behaviors, however, there was a trend ( $P = 0.10$ ) for euthanasia method by health status interaction for struggling bouts. Healthy pigs euthanized via HH experienced more struggling bouts than healthy pigs euthanized via CO<sub>2</sub> ( $P = 0.04$ ).

## Discussion

Measurements of the length of each treatment confirm that CO<sub>2</sub> is a quick method of euthanasia (AASV, 2008). There are many effects that are similar between the two treatments in this experiment. Both treatment groups exhibited decreased heart rates over time, increased blood gas levels and lactate concentrations and a decrease in power of EEG readings. Additionally, the different euthanasia methods elicited similar behavioral responses in both groups.

Higher concentrations of hemoglobin in pigs euthanized via HH are associated with exposure to high altitudes and a higher blood pH in hypobaric hypoxia pigs may be an example of the pig attempting to metabolically compensate to reach a more stable or neutral pH. Percent hematocrit is proportionate to the volume of blood occupied by red blood cells – a high percentage hematocrit in the blood is associated with pulmonary edema as in seen in hypoxia. Due to anaerobic metabolism, lactate values increase as the time without oxygen increases. Higher [glucose] and [LAC] in HH pigs may be attributed to differences in length of time the animals remained in the chamber thus altering concentrations of post-mortem metabolites.

Electroencephalogram data indicated that pigs euthanized via CO<sub>2</sub> reached a complete isoelectric state faster than pigs euthanized via HH indicating a more rapid death. However, pigs euthanized via CO<sub>2</sub> gasped more but had less bouts of paddling than pigs euthanized via HH. Furthermore, post-euthanasia percent hematocrit and hemoglobin, lactate concentrations, and pH were higher but *p*CO<sub>2</sub> was lower in HH compared to CO<sub>2</sub> euthanized pigs. A higher blood *p*CO<sub>2</sub> coupled with higher incidents of gasping indicates that CO<sub>2</sub> euthanized pigs may have experienced a greater amount of asphyxiation prior to death. However, pigs euthanized via HH had more lung lesions and HH was not effective in euthanizing every piglet which raises questions about its effectiveness as a consistent method of euthanasia. Further research utilizing HH at different altitudes and CO<sub>2</sub> at different induction rates may provide a more concrete solution to determining a low stress, humane method of on-farm euthanasia.

## References

1. American Association of Swine Veterinarians. 2008. On-farm euthanasia of swine. National Pork Board, Des Moines, IA, USA, document 04259–01/09.
2. Booth, Nicholas H. 1978. Effect of rapid decompression and associated hypoxic phenomena of euthanasia of animals: A Review. *Journal of American Veterinary Medical Association*.73: 308–314.

3. Dictionary.com “hypoxia.” Unabridged (v 1.1) Random House, Inc. 10 Nov. 2010. <Dictionary.com <http://dictionary.reference.com/browse/hypoxia>>.
4. Holladay, S. D. and F. W. Edens. 1987. Effect of cage density and rank in peck order on brain regional monoamines in adult *male Coturnix coturnix japonica*. *Comp. Biochem. Physiol.* 87:261–265.
5. MacGregor, R., W. Troxell and B. Rollin. 2008. Hypobaric hypoxia resulting from gradual decompression for a more humane method of mouse euthanasia. Thesis: Department of Mechanical Engineering Colorado State University.
6. Medical Dictionary On-line. “hypobaria.” Academic Medical Publishing & CancerWEB. 10 Nov. 2010. <Dictionary.com <http://dictionary.reference.com/browse/hypobaria>>.
7. Mullenax, C.H. and R.W. Dougherty. 1963. Physiologic responses of swine to high concentrations of inhaled carbon dioxide. *American Journal of Veterinary Research.* 24:329–332.
8. Panepinto, L., et al. 1983. A Comfortable, Minimum Stress Method of Restraint for Yucatan Miniature Swine. *Laboratory Animal Science* 33.1: 95.
9. Raj, A.B.M. and N.G. Gregory. 1995. Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Animal Welfare* 4:273–280.
10. Raj, A.B.M. and N.G. Gregory. 1996. Welfare implications of gas stunning of pigs: 2. Stress induction of anesthesia. *Animal Welfare.* 5:71–78.
11. Smith, D.C. 1965. Methods of euthanasia and disposal of laboratory animals. *Methods of animal experimentation.* Academic Press, New York and London. Vol. 1, Chapter 5, 167–195.
12. Shukitt-Hale, B., Banderet, L.E. and Lieberman, H.R. 1997. Elevation dependant symptom, mood and performance changes produced by exposure to hypobaric hypoxia. *Int J Aviat Psychol* 8(4):319–334.
13. Wittner, P.R. 2005. Transient hypoxia improves spatial orientation in young rats. *Physiological research.* 54:335–340.

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