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Comparison of CO2 versus mixed CO2: Argon Gas at Different Flow Rates Using the Smart Box Euthanasia Device as an Effective Method of Piglet Euthanasia

Larry J. Sadler
Iowa State University, ljsadler@iastate.edu

Chad D. Hagen
Value-Added Science and Technologies, LLC

Chong Wang
Iowa State University, chwang@iastate.edu

Tina M. Widowski
University of Guelph

Anna K. Johnson
Iowa State University, johnsona@iastate.edu

See next page for additional authors

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Authors
Larry J. Sadler, Chad D. Hagen, Chong Wang, Tina M. Widowski, Anna K. Johnson, and Suzanne T. Millman

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Comparison of CO₂ versus mixed CO₂:Argon Gas at Different Flow Rates Using the Smart Box Euthanasia Device as an Effective Method of Piglet Euthanasia

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Larry J. Sadler, Graduate Research Assistant, Department of Biomedical Sciences; Chad D. Hagen, Senior Vice President, Value-Added Science and Technologies, LLC; Chong Wang, Professor, Department of Veterinarian Diagnostic and Production Animal Medicine and Department of Statistics, Iowa State University; Tina Widowski, Professor, Department of Animal and Poultry Science, University of Guelph, CA; Anna K. Johnson, Associate Professor, Department of Animal Science, Iowa State University; Suzanne T. Millman, Associate Professor, Department of Veterinarian Diagnostic and Production Animal Medicine, Iowa State University

Summary and Implications

The objective of this study was to compare the effectiveness and response of weaned piglets to 100% CO₂ gas relative to a 50:50 CO₂:Argon gas mixture as an effective tool for euthanasia. A total of 180 piglets, BW 4.6 ± 0.7 kg, were utilized. Piglets were 16 to 24 days of age. Two gas mixtures (100% CO₂ and 50:50 CO₂:Argon) and 4 flow rates (slow, medium, fast, and prefill; 20%, 35%, 50%, and prefill with 20%, chamber volume per minute, respectively) were examined. Two piglets were placed in a modified Smartbox™ (Euthanex Corp, Palmer, PA) chamber, in which the lid and one side are composed of clear plastic to facilitate behavior observations. Piglets were scored using direct observation for latency to perform three behaviors associated with insensibility: loss of posture, last movement and gasping. Open mouth breathing occurred prior to insensibility and was used as an indicator of distress. The CO₂:Argon gas mixture and slow flow rates prolonged the duration of insensibility, as measured by last movement and did not confer advantages for measures of distress.

Introduction

The U.S. swine industry euthanizes piglets when their chances of survival are low and they are suffering due to injury or illness. This results in million of piglets being euthanized annually, and tools are needed to accomplish euthanasia quickly, economically and safely, as a repeatable humane process.

Carbon Dioxide (CO₂) gas to euthanize young pigs is acceptable by National Pork Board guidelines and is increasingly common. CO₂ is economical, relatively safe and readily available. CO₂ is a colorless, odorless gas, which stuns by lowering the pH of the central nervous system. It is capable of doing this because it is mildly acidic. However, this acidity may cause sensation and distress. Argon has been proposed as a more humane alternative, since it is unreactive throughout the body’s systems, stunning through asphyxiation. Evidence from other species suggests that argon may be less aversive than the standard CO₂ methods. Furthermore, little empirical research exists to support best management practices for on-farm CO₂ euthanasia, in terms of gas flow rate, concentration or duration of exposure. Therefore the objective of this study was to compare the effectiveness and response of weaned piglets to 100% CO₂ gas relative to a 50:50 CO₂:Argon gas mixture as an effective tool for euthanizing.

Materials and Methods

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee (11-09-6825-S). The experiment was conducted from May to September, 2010.

Animals and housing: A total of 180 mixed sex piglets (90 barrows and 90 gilts) were used from commercial PIC genetic lines. Piglets were obtained and housed at the Iowa State University Swine Nutrition Farm. Piglets weighed 4.6 ± 0.7 kg and were 16 to 24 days of age.

Experimental design: Piglets were compared as mixed sex pairs. The experimental design for this study was a 2 x 4 factorial arrangement of treatments. Two gas mixtures: 100% CO₂ (CO₂) and 50:50 CO₂:Argon (CA) with four different chamber exchange rates: slow, medium and fast, allowing 20%, 35%, 50% chamber volume turnover per minute respectively, and pre-fill of the chamber with 20% chamber volume turnover per minute.

Euthanasia protocol: Piglets were placed into a plastic chamber (inside dimensions 43 wide, x 60 long, x 30 height, cm), with 2 clear sides facilitating behavior observations. The floor was fitted with a black rubber mat to prevent slipping. Gas was supplied utilizing a Euthanex AgPro™ (V-ast, Mason City, IA; Figure 1) and a constant gas flow was provided by a compressed gas regulator (Western Enterprises, Westlake, OH). Between each treatment the chamber was blown out with ambient air.
Behavioral measures: Piglets were observed directly for latency to behavioral indicators of stunning and death (Table 1).

Table 1. Behavioral indicators of stunning and death.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Mouth Breathing (OMB)</td>
<td>Piglets mouth is open, taking in quick breaths, with distinct thoracic movements; panting; upper and lower jaw being held open with the top lip pulled back, exposing gums or teeth and panting (pronounced inhalation and exhalation observed at the flanks)²,³</td>
</tr>
<tr>
<td>Gasping (GASP)</td>
<td>Rhythmic breaths characterized by very prominent and deep thoracic movements, with long latency between, may involve stretching of the neck; often occurs right before or after loss of posture¹,²</td>
</tr>
<tr>
<td>Loss of posture (LP)</td>
<td>Piglet is slumped down, making no attempt to right itself, follows a period of attempts to maintain posture; loss of attitude of position of the body</td>
</tr>
<tr>
<td>Last movement (LM)</td>
<td>No movement is observed by the piglet of any type</td>
</tr>
</tbody>
</table>

Statistical analysis: Analysis was performed in SAS. OMB, GASP, and LP were analyzed as Univariate product-limit estimation of the survival curves. LM data was log transformed and analyzed as a mixed model with fixed effects of sex and treatment, and blocked by day of treatment. Raw means were calculated using Proc Means.

Results and Discussion
When comparing gas types, differences were observed for LM with CA treatment taking longer relative to CO₂. Differences were not observed for OMB, LP or GASP between the two gas types. Within gas types, as expected, gas flow rate significantly (P < 0.001) affected LM, with the slow flow rate taking longer than the medium, fast, or prefill. No differences were observed for all other reported measures. In conclusion, CA and slow flow rate prolonged the duration of insensibility, as measured by LM and did not confer advantages for measures of distress (OMB).

Table 2. Latency to last movement by gas type and flow rate; P-value within gas type over flow rates P<0.003.

<table>
<thead>
<tr>
<th>Gas Mixture</th>
<th>Flowrate</th>
<th>CO₂</th>
<th>SE</th>
<th>CA</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td></td>
<td>529</td>
<td>181</td>
<td>774</td>
<td>216</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>312</td>
<td>40</td>
<td>467</td>
<td>37</td>
</tr>
<tr>
<td>Fast</td>
<td></td>
<td>274</td>
<td>27</td>
<td>397</td>
<td>32</td>
</tr>
<tr>
<td>Prefill</td>
<td></td>
<td>269</td>
<td>73</td>
<td>451</td>
<td>209</td>
</tr>
</tbody>
</table>

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Corresponding author: Suzanne Millman; phone: 515-294-2817; email: smillman@iastate.edu