RESEARCH REPORT

ANIMAL WELFARE

Title: Field evaluation of humane pig euthanasia using a dump trailer as a CO2 chamber-**NPB #20-119**

Investigator: Marisa Rotolo

Institution: Pig Improvement Company

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Industry Summary:
In the summer of 2018, African swine fever virus was detected in China, highlighting the need renewed preparation efforts in the United States against foreign animal diseases. Since 2018, many efforts have focuses on improved surveillance, response by federal and state animal health officials as well as identification of gaps in the swine industry in the event of a FAD incursion on US soil. One of these gaps was methods to successfully perform mass depopulation of swine herds in such a way that is efficient, safe, welfare-friendly and does not cause undue emotional strain on farm staff. One method of mass depopulation that took center stage during the COVID 19 pandemic was the use of carbon dioxide gas as a means of mass depopulation. Due to staff shortages at processing plants, populations of pigs were euthanized. In most cases the method of euthanasia was carbon dioxide. Despite the documented efficacy of this method, pig level variables such as movement, vital signs, and temperature had not been explored. These variables are quintessential to understanding and ensuring that the animal welfare aspects of a mass depopulation method fall within the AVMA Guidelines for Depopulation of Animals. Therefore, the objective of this work was to evaluate the effectiveness and animal welfare implications of administering the inhalant anesthetic, carbon dioxide (CO_2) in a 12-foot dump trailer as a method of humane euthanasia for sows, grower and market weight pigs. In brief, a 12-foot dump trailer was modified into a CO2 chamber for this study. CO2 was delivered into the trailer through a 100-foot tube attached to a vaporizer, receiving CO2 from a CO2 tank. Groups of pigs were moved into the trailer and administered CO2 at a rate of 20-30% of chamber volume per minute over a 5-minute wash in period. After 15 minutes, pigs were assessed for confirmation of death. Oxygen and CO2 levels were monitored throughout the entire trial period. No secondary means of euthanasia was necessary. The first two groups consisted of 3 sows each which received sedatives to ensure that the process worked without causing undo stress on the animals. Two groups of grower pigs, two groups of sows and one group of market weight animals

were successfully euthanized using the CO2 trailer. In each group, three animals were monitored for movement using a HOBO-Pendant G accelerometer and heart rate using Cardiac Insight ECG. Results from these tools indicate that cessation of movement occurred within 282 seconds as an average across all groups and cessation of heartbeat was confirmed in all groups. The observations from this study support the use of CO2 as a method of mass depopulation due to the ease of use, animal welfare implications, ability to perform with large groups of animals, human safety and efficiency.

Key Findings:

- Dump trailer can be successfully modified for use as a CO2 chamber
- CO2 inhalant anesthetic can be used to successfully euthanize large groups of pigs of multiple sizes
- Cessation of movement occurred on average across multiple age groups within 282 seconds after exposure to CO2.

Keywords: carbon dioxide, mass depopulation, vaporizer

Scientific Abstract:
For the veterinary community, the American Veterinary Medical Association (AVMA) maintains ownership of the Guidelines for Depopulation of Animals (AVMA). Methods of euthanasia for swine include gunshot, captive bolt, CO2, anesthetic overdose and blunt force trauma (age-dependent) (AVMA). Due to the threat of foreign animal disease, the swine industry has renewed interest in methods of mass depopulation. One of these methods is the use of carbon dioxide as an inhalant anesthetic. Research has supported the use of carbon dioxide as a means of euthanasia if the gas is delivered at a rate of 20-30% per minute over the course of five minutes. While carbon dioxide has long been used on a small scale within swine farms, the use of this gas for euthanasia of large groups of animals and the welfare implications has not been explored. In this study, the objective is to evaluate the effectiveness and animal welfare implications of administering the inhalant anesthetic, CO2, in a 12-foot dump trailer as a method of humane euthanasia for sows, grower and market weight pigs. A 12-foot dump trailer was converted into a swine euthanasia chamber. Carbon dioxide was introduced into the trailer via a vaporizer connected to a carbon dioxide tank. Four trials were performed with sows, grower and market weight animals. Trial 1 was split into two groups of 3 sows weighing approximately 450 lbs each. Trial 2 was split into one group of fifteen 80 lb pigs and one group of twenty 80 lb pigs. Two groups of 5 sows at approximately 450 lb were euthanized in Trial 3. Seven market weight pigs (~250 lb) were euthanized in Trial 4 which consisted of one group. In each group, with the exception of trial 4, 3 animals were fitted with single use ECG devices and accelerometers as described previously. Six animals were fitted with these devices in Trial 4. Each device was activated just prior to loading into the chamber. Mean time to cessation of movement (COM) across all three size categories (grower, finisher, sows) was 282 seconds (95% CI 245 – 320). No significant differences in time to COM were detected between size categories (p=0.9742, Kruskal-Wallis rank test) (non-normal distribution per Shapiro-Wilk test: p=0.03076). ECG tracing data were available for 12 individual pigs across the 4 trials. Asystole was noted in all the tracings. Persistent electrical activity (PEA) was noted following presence of arrhythmias in 10 of the 11 complete tracings. PEA slowly decreased in intensity until it was no longer detectable by the end of the 15-minute post-CO2 shutoff period for 7 of

8 tracings. CO2 levels of 80% or greater were reached within 5 minutes of introduction into the chamber for all groups in Trials 1-4. The data found in this study support the use of carbon dioxide for a method of mass depopulation of swine herds.

Introduction:

In the face of mounting foreign animal disease (FAD) threats, the United States swine industry has renewed focus on preparation efforts in the event of a positive detection of any of the three FADs, African swine fever (ASF), Classical swine fever (CSF) and foot and mouth disease (FMD). Since the detection of ASF in China in 2018, many efforts are geared toward managing an outbreak of ASF within the United States. If ASF, or any of the FADs were to be detected, the main flow of events would be focused on detection, containment and eradication. Any herd with a positive diagnosis of ASF would undergo mass depopulation to limit spread of the virus referred to as "stamping-out" (USDA, 2020). This approach is also outlined in the OIE Terrestrial Animal Health Code and affects animals with clinical signs and those that are suspected of being positive (OIE). Mass depopulation should be performed in a humane, safe and efficient manner. For the veterinary community, the American Veterinary Medical Association (AVMA) maintains ownership of the Guidelines for Depopulation of Animals (AVMA, 2020). Methods of depopulation for swine include gunshot, captive bolt, CO2, anesthetic overdose and blunt force trauma (age-dependent) (AVMA, 2020).

Due to the ability to depopulate large groups of pigs, CO2 has garnered multiple research studies as a tool for mass depopulation of swine (Arruda et al., 2020). Research has shown that CO2 inhalation results in respiratory acidosis leading to decreased intracellular pH and death as well as providing analgesic and anesthetic effects (Meyer et al., 2005). While combinations of gases have been evaluated for humane euthanasia, such as nitrogen and CO2, CO2 has been demonstrated to have a faster loss of consciousness before pain receptors are activated, leading some to believe that it is a more humane inhalant for depopulation (Meyer et al., 2005).

Coupled with renewed efforts for FAD preparedness and response, the COVID-19 global pandemic created circumstances in which mass depopulation became a reality for producers. Disruption in the market due to quarantines, reduced output within packing and processing plants, decreased demand of meat products from commercial restaurants led to an overpopulation of pigs with limited housing space (Arruda et al., 2020). CO2 chambers were quickly built for use as mass depopulation methods for overpopulated producers. Past research has shown that a standard dump trailer can be converted into a swine euthanasia chamber (Rice et al., 2014). A euthanasia chamber should aim to have no air leaks, a delivery system for the CO2, and a cover (Rice et al., 2014). For this study, a 12-foot dump trailer was converted into a swine euthanasia chamber for evaluation of a method of mass depopulation.

Objectives:

1. Evaluate the effectiveness and animal welfare implications of administering the inhalant anesthetic, CO2, in a 12-foot dump trailer as a method of humane euthanasia for sows, grower and market weight pigs
    a. Identify changes in animal behavior, vitals and/or time required to induce loss of consciousness and death

Materials & Methods:

Phase 1-Conversion of 12-foot dump trailer to swine euthanasia chamber
a 12-foot dump trailer was modified by adding approximately 1ft wooden boards to all sides of the trailer. A custom wooden roof with metal fencing was added to the trailer. A clear polymer sheet was stapled to the roof to improve light penetration for the animals while still allowing the trailer to be sealed to limit CO_2 dissipation. Two hinges were added to the roof to allow it to be lifted to help encourage animal movement on to the trailer. Welded channels were added to the back of the trailer to allow for utilization of a quarter inch white board for temporary closure of the trailer for animal containment as well as addressing the gap between the trailer back gate and roof resulting from the addition of wooden boards to the sides of the trailer. The final dimensions of the trailer were 6ft (1.8288m) x 10 ft (3.048m) x 4 ft (1.2192m). The total volume of the trailer was approximately 6.796m^3. A 100ft tube was attached to the vaporizer and placed through a hole in the white board to allow for introduction of CO_2.

Delivery of CO2 into swine euthanasia chamber
A high pressure 1,000L Perma-Cyl bulk Co_2 tank delivered gas to a 300lb vaporizer (Thermax, Inc., North Dartmouth, MA, USA). CO_2 was introduced into the trailer via a 100ft tube that connected to the vaporizer. A flowmeter was used to control the rate of CO_2 introduction. Rates varied based on stocking density within the trailer between 35 and 48 SCFM. CO_2 was delivered at a rate of 30% per minute over 5 minutes to achieve a minimum concentration of 63% CO_2. After five minutes the gas was shut off and the trailer was monitored for 15 additional minutes. Throughout the process, CO_2, O_2 and temperature were monitored every minute and recorded. CO_2 and O_2 levels were monitored using a GasLab® Pro Multi-Gas Sampling Data Logger (CM-1000, CO2 Meter, Ormond Beach, FL, USA).

Phase 2-System Test
After installation of the system, a trial run was completed without pigs to verify appropriate system function. CO_2 and O_2 monitors verified that appropriate CO_2 levels were reached in the swine euthanasia trailer. To adhere to AVMA guidelines, CO_2 was introduced at a flow rate of 30% of chamber volume per minute over a 5-minute period. The chamber volume of the dump trailer is approximately 6.796m^3. At 20°C and 1 atmosphere, CO_2 occupies approximately 0.55m^3 per kilogram (kg). This translates to 12.356 kg of CO_2 per trailer load or a flow rate of 2.47 kg CO_2 per minute. The CO_2 and O_2 sensors will confirm the correct concentration of CO_2. After the 5-minute wash-in period, CO_2 and O_2 levels were monitored for 30 minutes to check the system for leaks and ensure maintenance of CO_2 levels.

Phase 3: Evaluation of the system for humane euthanasia of sows, nursery and market weight pigs by CO_2
Four field trials were conducted and separated out by age and weight of the animals. Trial 1 was performed with anesthetized animals while the remainder of the trials were performed with non-anesthetized animals. Trial 1 was split into two groups of 3 sows weighing approximately 450 lbs each. Trial 2 was split into one group of fifteen 80 lb pigs and one group of twenty 80 lb pigs. Two groups of 5 sows at approximately 450 lb were euthanized in Trial 3. Seven market weight pigs (~250 lb) were euthanized in Trial 4 which consisted of one group.

Three sows from each group for Trial 1 received an intramuscular injection of 5 ml of telazol, ketamine and xylazine mixture. This equated to 500mg of telazol, 250mg ketamine and 250mg xylazine. The sows received the injection just prior to loading into the chamber.

In each group, with the exception of trial 4, 3 animals were fitted with single use ECG devices and accelerometers as described previously. Six animals were fitted with these devices in Trial 4. Each device was activated just prior to loading into the chamber.

Once animals were safely loaded onto into the chamber and the chamber was closed, CO2 was introduced at a rate of 30% per minute over a 5-minute period. At 5 minutes, the CO2 was shut off and the chamber was monitored for an additional 15 minutes. At the end of the 20-minute period, the roof of the chamber was opened, and confirmation of death was performed by veterinary personnel once O2 levels reached >19%.

Results:

Carbon dioxide (CO2), oxygen (O2), and temperature monitoring

CO2 levels of 80% or greater were reached within 5 minutes of introduction into the chamber for all groups in Trials 1-4 with a corresponding decrease in O2 levels to < 6% (Figure 1&2). O2 levels returned to $\geq$ 19.8% within 5 minutes or less within the chamber once the roof of the chamber was opened (mean, 3.4 minutes).

Chamber temperature near the CO2 inlet ranged from 88.1-126.8°F, and away from the inlet ranged from 80-113°F (data across all trials).

Animal monitoring

Time at cessation of movement (COM) was determined from distal limb HOBO accelerometer devices. Movement was not assessed in anesthetized sows (Trial 1). Mean time to COM in conscious grower pigs (Trial 2) was 275 seconds (95% CI 228 – 322). Mean time to COM in conscious sows (Trial 3) was 304 seconds (95% CI 216 – 392). Mean time to COM in conscious finisher pigs (Trial 4) was 267 seconds (95% CI 214 – 319). Mean time to COM across all three size categories (grower, finisher, sows) was 282 seconds (95% CI 245 – 320). No significant differences in time to COM were detected between size categories ($p=0.9742$, Kruskal-Wallis rank test) (non-normal distribution per Shapiro-Wilk test: $p=0.03076$).

ECG tracing data were available for 12 individual pigs across the 4 rounds of experiments (Table 1). One tracing (Trial 1, Group 1, Pig ID 1) ends before any significant cardiac electrical abnormalities were noted. Asystole was noted in all the remaining 11 tracings. Persistent electrical activity (PEA) was noted following presence of arrhythmias in 10 of the 11 complete tracings. PEA slowly decreased in intensity until it was no longer detectable by the end of the 15-minute post-CO2 shutoff period for 7 of 8 tracings. Several tracings stop before the end of the 15-minute hold.

Discussion:

The objective of this study was to evaluate the effectiveness and animal welfare implications of administering the inhalant anesthetic, CO2, in a 12-foot dump trailer as a

method of humane euthanasia for sows, grower and market weight pigs. CO2 has been established as an AVMA approved method of depopulation. As an inhalant gas anesthetic, CO2 is inexpensive, can be used safely by people in well ventilated areas, and has analgesic effects (Kinsey et al., 2016). Given the ease of converting dump trailers into CO2 swine euthanasia chambers, the use of CO2 as a method of mass depopulation has increased with in the swine industry due to constraints of COVID-19 and is a viable option in the event of a FAD incursion in the United States.

In this study, a 12-foot dump trailer was converted into a swine euthanasia chamber to assess the use and effectiveness of this method as a means of depopulation. Four trials were conducted to determine the efficacy and animal welfare implications of this method. As seen in the results, accelerometer data revealed that cessation of movement occurred within 282 seconds across all four trials. The data collected by the ECG monitors supported confirmation of death through presence of asystole. This data supports the use of CO2 as a humane method of depopulation.

In addition to individual animal data, temperature was also monitored within the chamber. As seen in the results, the temperature within the chamber varies and is something to take into consideration for veterinarians. The wide ranges of temperatures possibly reflect difference in overall mass of animals loaded into the trailer for individual rounds and/or variation in temperature of CO2 gas entering the chamber following vaporization. Given the high observed temperatures, this method should be employed in cooler temperatures to prevent animal stress due to overheating.

Monitoring of CO2 and O2 levels supports the safety of this method for farm staff. Return to normal environmental O2 levels occurred quickly, on average 3.4 minutes after the chamber was opened. As a safety precaution, O2 monitors should be used in conjunction with these chambers to ensure that levels are safe for people prior to confirming death in the animals. Monitoring of CO2 also revealed that animal density within the chamber affects the flowrate necessary to reach the AVMA requirement of CO2 delivery of 20-30% per minute over a five-minute time span. As the size of the groups increased, the flowrate could be decreased, and adequate CO2 levels were achievable.

Given the results of this work and the research of others, the use of a converted dump trailer as a CO2 swine euthanasia chamber allows for efficient and animal-welfare friendly method of depopulation of large groups of pigs.

References

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Table 1. Presence of fatal rhythms and PEA* in individual ECG tracings during CO2 depopulation.

Trial	Group	Pig ID	Atrial standstill	Ventricular fibrillation	Asystole	Ventricular complexes or PEA present after fatal rhythm?	PEA at end of 15m hold?
1	1	1				.	.
1	1	2	X		X	Yes	No
1	1	3	X		X	Yes	No
1	2	5	X		X	Yes	.
1	2	6			X	Yes	.
3	1	1	X	X	X	Yes	Yes
3	1	3		X	X	Yes	No
3	2	4			X	Yes	No
3	2	6	X		X	Yes	No
4	1-2	2			X	No	No
4	1-2	3			X	Yes	No
4	1-2	5	X		X	Yes	.

*PEA = persistent electrical activity following clinical asystole (i.e. cessation of heart contraction/pulse)

Figure 1. Percent carbon dioxide (CO2) in chamber during euthanasia of sows (circles) growers (squares) and finishers (triangles)..

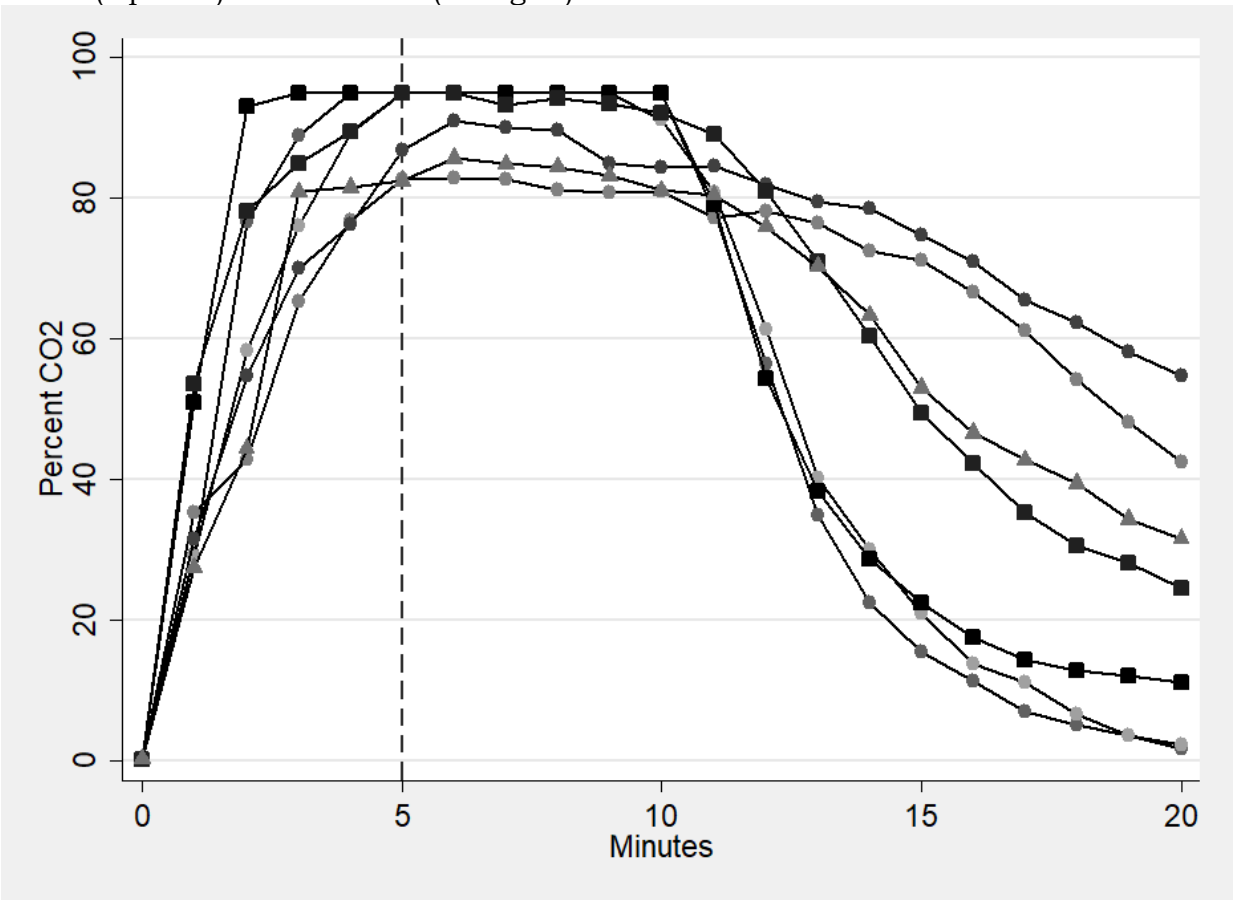


Figure 2. Percent oxygen (O2) in chamber during euthanasia of sows (circles) growers (squares) and finishers (triangles).

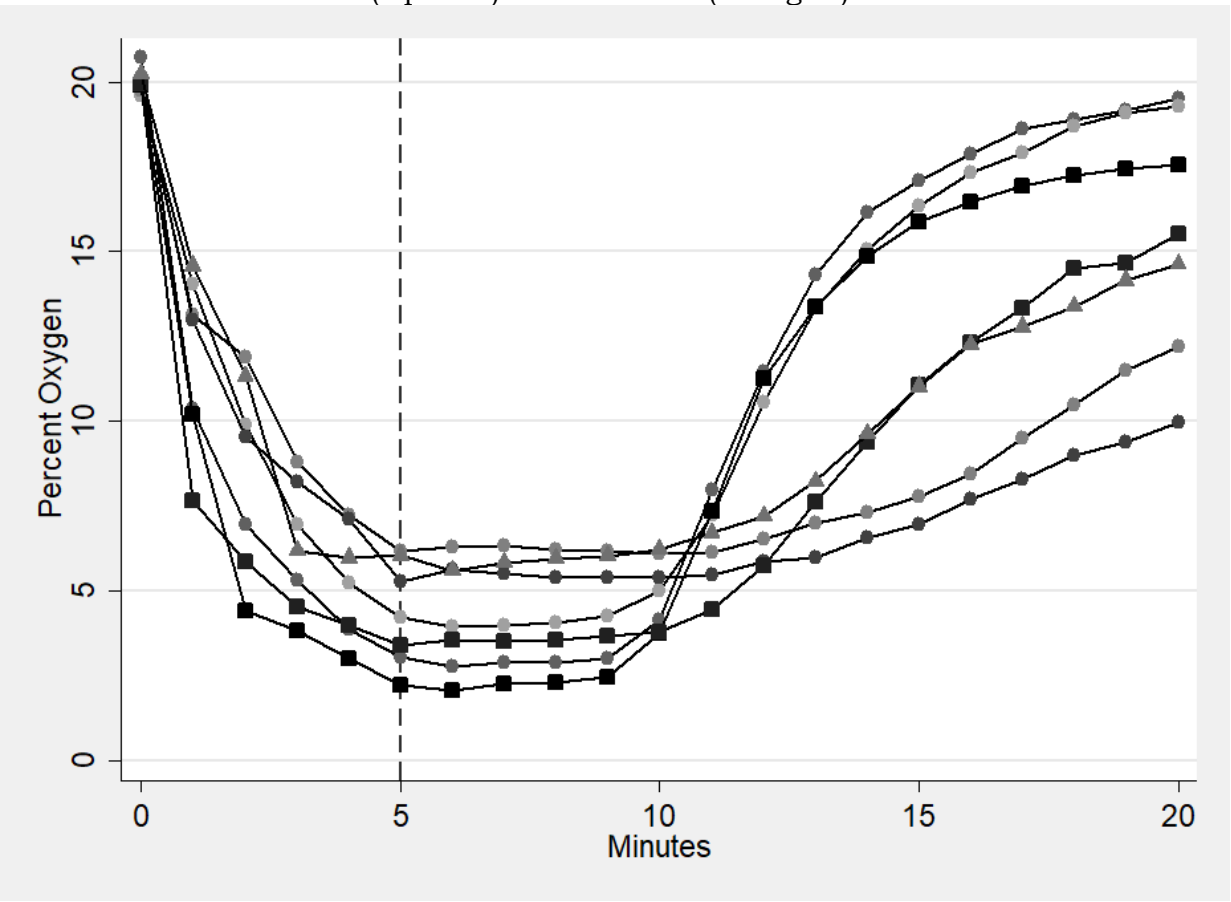
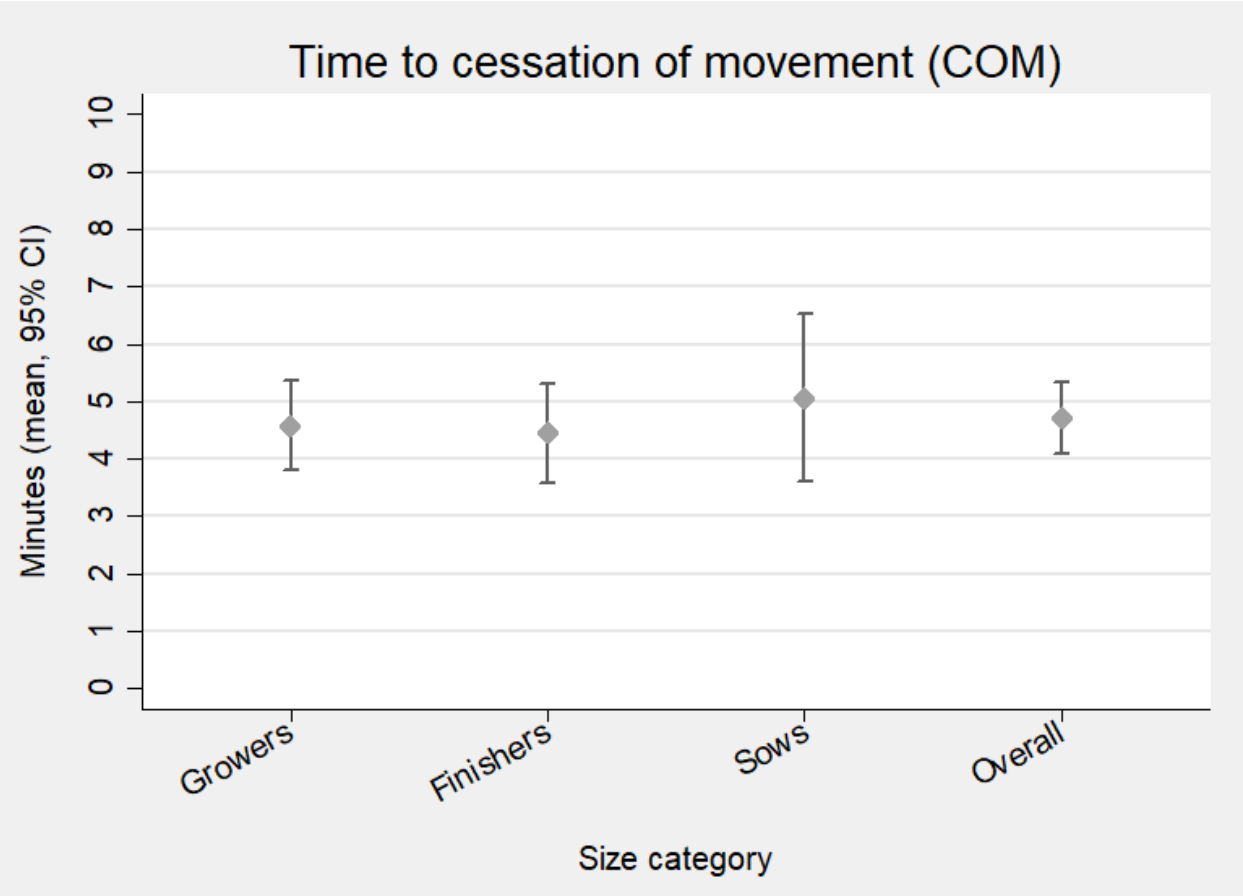


Figure 3. Mean time to cessation of movement (COM) following the start of carbon dioxide gas introduction is shown for grower pigs (n=5), finisher pigs (n=6), sows (n=6), and overall (n=17). Mean (diamond) and 95% confidence interval (spikes) are shown. Time to COM was determined using HOBO G pendant accelerometers fitted to individual animals.



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