EMERGENCY DEPOPULATION OF CAGED LAYER HENS USING A COMPRESSED AIR FOAM SYSTEM AND CO_2 GAS

by

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ABSTRACT

Outbreaks of avian influenza (AI) and other highly contagious poultry diseases continue to be a concern for those involved in the poultry industry. In the situation of an outbreak, emergency depopulation of the birds involved is necessary. Emergency responders have to select the best depopulation method that minimizes the spread of the disease and risks to human health but also addresses animal welfare concerns. The determination of time to unconsciousness is very important when selecting a depopulation method, as it shows when the birds are no longer aware of their surroundings or in any pain.

The purpose of this experiment was to evaluate the efficacy of a compressed air foam system (CAFS) against traditional CO_2 gassing in mass emergency depopulation of caged layer hens. The experiment was conducted using a randomized block design with commercial layer hens exposed to one of three randomly selected depopulation treatments: CAFS, CAFS with CO_2 gas, and CO_2 gas alone. The time to unconsciousness, brain death, and terminal convulsions were recorded for each bird. Unconsciousness and brain death were evaluated using the EEG signals recorded from a wireless transmitter surgically implanted into the brain of the bird. Terminal convulsions were determined through analysis of recorded data from an accelerometer attached to the layer's leg during depopulation. Critical time for physiological events were extracted from the EEG and accelerometer data and were compiled in Excel and statistical analysis was performed using JMP. Statistical analysis methods included Fit

Y by X analysis using ANOVA and a student's t test of means. All tests were conducted at the 5% ($\alpha = 0.05$) significance level.

There was a statistically significant difference in the time to unconsciousness between the two compressed air foam methods and the CO_2 gassing. However, there was no statistically significant difference when comparing the two foams. CAFS with CO_2 gas was the fastest treatment with respect to unconsciousness (μ=16.9 sec) with regular CAFS close behind it (μ= 19.5 sec). CO_2 gas was significantly slower (μ= 38.5 sec). The time to brain death of the birds show there was no statistically significant difference between CAFS (μ= 131.1 sec), CAFS with CO_2 gas (μ= 135.5 sec), and CO_2 gas (μ= 142. 4 sec). The time to terminal convulsions of the birds showed that there was no statistically significant difference in the time to motion cessation for CAFS (μ= 211.4 sec), CAFS with CO_2 gas (μ= 224.0 sec), and CO_2 gas (μ= 226.4 sec).

The results of this experiment show that a compressed air foam system was able to depopulate layer hens housed in cages and was more rapid at causing unconsciousness than CO_2 gas. Though not statistically significant, the compressed air foam system caused brain death and motion cessation faster than CO_2 gas. The time to unconsciousness was also more consistent for the two foam treatments, with less variation from the mean compared to CO_2 gas. This information may play a role in how organizations such as the American Veterinary Medical Association (AVMA) and US Department of Agriculture (USDA) evaluate the suitability of a compressed air foam system for mass depopulation of caged layer hens.

Chapter 1

REVIEW OF LITERATURE

Avian diseases such as exotic Newcastle disease, highly pathogenic avian influenza (HPAIV), and others continue to be a concern for the poultry industry today. There is currently an outbreak of avian influenza occurring in the Midwestern United States in Minnesota and Iowa that researchers are calling the worst bird flu epidemic in the nation's history. So far they have experienced losses of 5.8 million turkeys in Minnesota and 26 million birds in Iowa, 23 million of which are of the type that lay eggs (Sreenivasan, 2015). The approach for dealing with such contagious diseases includes surveillance, quarantine, depopulation, disposal, and decontamination. Depopulation of the diseased flock minimizes animal suffering and stops virus replication and dissemination. The American Veterinary Medical Association (AVMA) has outlined the animal welfare standards for both general euthanasia and depopulation during outbreaks. Euthanasia methods for poultry (domesticated birds used for egg, meat, or feather production [eg, chickens, turkeys, quail, pheasants, ducks, geese]) include gas inhalation, manually applied blunt force trauma, cervical dislocation, decapitation, electrocution, gunshot, captive bolt, and injectable agents (AVMA, 2013). The 2013 AVMA Guidelines on Euthanasia stated that they were preparing a separate document detailing acceptable practices where depopulation is deemed necessary, however, they have not yet released this document. The 2007 AVMA Guidelines on Euthanasia include depopulation and state, "Under unusual circumstances, such as disease eradication and natural disasters, euthanasia options

may be limited. In these situations, the most appropriate technique that minimizes human and animal health concerns must be used" (AVMA, 2007). An outbreak of HPAIV in caged layers is one of the worst-case scenarios for the current poultry industry. The cages present in layer houses present significant difficulty where depopulation is concerned because the birds either need to be removed prior to depopulation or immediately after depopulation, significantly increasing the labor and time requirements. Currently there is no depopulation method that is effective or fast enough to efficiently prevent the spread of disease.

There are four different types of gas depopulation available in poultry, all of which fall under the Acceptable with Conditions category in the 2013 AVMA Guideline on Euthanasia. The conditions for euthanasia by inhaled agents include that birds should be checked to verify death because they may appear dead but can regain consciousness if the exposure time or the concentration of the agent is insufficient. Gases must be supplied in purified forms without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The gas-dispensing system should have sufficient capacity and control to maintain the necessary gas concentrations in the container being utilized, and the container itself should be sufficiently airtight to hold the gas at appropriate levels (AVMA, 2013). The four types of gassing approved for depopulation in poultry include carbon dioxide (CO_2), carbon monoxide (CO), nitrogen (N), and argon (Ar). All of the gasses have the potential to cause involuntary motor activity in birds such as flapping of the wings or other terminal convulsions, which can damage tissues and be disconcerting for observers. (AVMA 2013)

CO_2 is one of the most common gassing methods used for depopulation of poultry. Carbon dioxide acts by directly affecting the respiratory system of the birds in contrast to other gases like argon (Ar) and nitrogen (N_2) that work by displacing the oxygen in the environment causing hypoxia (van den Bogaard,1985 in (Gerritzen *et al.* 2000). Gassing provides significant logistical complications for depopulation. Gerritzen has reported that the CO_2 levels for large-scale depopulation of broilers must exceed 30% in combination with a residual O_2 level below 13% and that these levels must be maintained for 30 min to ensure that death occurs in all the birds (Gerritzen et al. 2004). If the house being gassed experienced any structural damage or is not structurally sound, this only increases the difficulty of gassing as it creates openings through which the gas can escape, making it harder to achieve the level needed for effective depopulation. An additional concern raised with CO_2 gassing is the drop in temperature caused by the introduction of CO_2, which is another welfare concern where whole house gassing is involved. The plume of vaporized liquid CO_2 can extend up to 10 m and can be as cold as -79° C, which is the sublimation temperature of the CO_2 at atmospheric pressure (Ryan et al. 2006). During the application of the gas, the birds are exposed to the gas and temperature drop before the gas reaches unconsciousness levels (20% CO_2), (Gerritzen *et al.* 2006, 39-42) or lethal levels (30% CO_2), (Gerritzen *et al.* 2004). Carbon dioxide gas causes an unpleasant sensation during the inhalation of the gas and the birds exhibit gasping, vocalization and headshaking during the induction of unconsciousness (Raj 1996). This behavior is opposite of what is experience when using argon gas. Argon is an inert gas with no taste or odor, is not detected by the birds and they exhibited no stress behaviors or signs of respiratory distress before they lose consciousness (Raj 1996). Other sources,

including Alphin *et al.* (2010), document that field use of Ar - CO_2 gas mixtures may take significantly longer and result in more variability than CO_2 gas.

A new alternative for depopulation of poultry, water-based foam, was developed after the 2004 Delmarva LPAIV H7N2 outbreak. The polyethylene tent method of depopulation proved to be too difficult and, as a result, a new method of depopulation was desperately needed. The process, which was initially designed for floor-reared poultry, uses foam generation equipment to cover the birds with a blanket of modified firefighting foam. The immersion in the foam causes a rapid blocking of the airway, causing mechanical hypoxia, resulting in the cessation of heart activity (Benson *et al.* 2007). The foam was tested both with and without CO_2 present in the bubbles formed, and was compared to standard CO_2 gassing methods in terms of time to heart activity cessation. The differences in the foam times with and without the CO_2 present were not significant, indicating that the presence of the gas did not affect ECG cessation time (Benson et al. 2007). Foam allows a safer substitute for depopulation over CO_2 gas. It can be used in damaged on structurally unsound houses and poses less of a threat to the workers involved.

While the AVMA Guidelines on Euthanasia did not contain a section on depopulation, the 2015 AVMA website does include a section on poultry depopulation. The AVMA supports the use of water-based foam as a method of mass depopulation in accord with the conditions and performance standards outlined by the US Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) (AVMA, 2015). The condition are as follows: 1) Appropriate method of depopulation for floor-reared poultry 2) Animals are potentially infected with a zoonotic disease 3) Animals are experiencing an outbreak of a rapidly spreading

infectious disease that, in the opinion of state or federal regulatory officials, cannot be contained by conventional or currently accepted means of depopulation, and 4) Animals are housed in structurally unsound buildings that would be hazardous for human entry, such as those that may result from natural disaster (AVMA, 2015).

The objective of this study was to evaluate different methods of depopulation for layer hens using a novel type of foam and CO_2 gas. The efficiency was evaluated using the electrocardiogram (ECG), the electroencephalogram (EEG) and the accelerometers to measure time to measure time to physiological events during depopulation. EEG was used to determine the time to loss of consciousness and brain death in the birds. Acceleration was used to measure time to terminal convulsions, which is tied to brain death. This study was conducted to simulate the challenges of an outbreak in a layer hen facility.

Chapter 2

METHODS AND MATERIALS

The purpose of this experiment was to evaluate the efficacy of a compressed air foam system (CAFS) with and without CO_2 gas against traditional CO_2 gassing in mass emergency depopulation of caged layer hens. The experiment was conducted using a randomized block design with commercial layer hens exposed to one of three randomly selected depopulation treatments: CAFS, CAFS with CO_2, and CO_2.

A total of 180 spent layer hens (birds > one year of age) were used for this experiment. Out of the 180 birds, 48 were surgically instrumented with a wireless EEG transmitter. The surgery birds were also outfitted with an electrocardiogram (ECG) and accelerometer. A total of 3 data sets were collected for each of the 48 surgery birds, comprising a total of 144 readings collected. For each surgery bird there was also a companion bird located in the same cage and two additional birds located in an adjacent cage. These three birds were all outfitted with an accelerometer and data was collected for each resulting in an additional 144 data sets collected. There were 16 surgery birds for each treatment. The first four trials did not contain any companion birds or additional birds fitted with accelerometers and thus they were removed from the data set. After removal of these trials there were 15 surgery birds for the CAFS treatment, 14 surgery birds for CAFS with CO_2 gas, and 15 surgery birds for CO_2 gassing. All testing was performed under the approval and guidelines of the University of Delaware Agricultural Animal Care and Use Committee (Protocol (33) 02-24-14R,

Revised 06-03-14) and followed guidelines laid out by the Federation of Animal Science Societies (Federation for Animal Science Societies, 2010).

Approximately 24-48 h before a trial, four birds were randomly selected from the University of Delaware flock. Each bird was anesthetized using 5% isoflurane (IsoSol; Vedco, Inc., St. Joseph, MO), was intubated and then placed on 3% isoflurane for maintenance of anesthesia. Birds were given a non-steroidal anti-inflammatory drug injection (carprofen or meloxicam) after intubation to allow for time for the medicine to start working before surgery was complete. Three channel wireless biopotential transmitters (PhysioTel model F50-EEE, Data Sciences, International, St. Paul, MN) were surgically implanted in the back of the neck. Three leads (two recording leads and one ground lead) were placed on the meninges covering the telencephalon through 0.9 mm holes that were drilled into the parietal bone, two holes on the right side of the midline and one on the left, using a high speed microdrill (model 18000-17, Fine Science Tools, Foster City, CA). One recording lead was placed on each side of the midline and the ground lead was place on the right side. Two leads were implanted in the *complexus* muscle just below the skull for electromyography (EMG). All leads were held in place with cyanoacrylate. After surgery the birds were allowed to recover for a period of 24 h. The surgical procedure is based on Savory and Kostal (1997, 2006) and has been used with broilers (Alphin et al., 2010), turkeys (Rankin, 2010), layers, and ducks Caputo et al., (2012, 2013).

On the following day after surgery the four surgery birds underwent a randomly selected treatment (CO_2, CAFS, or CAFS w/ CO_2). Immediately before depopulation, electrocardiogram (ECG) electrodes were placed on the right wing and both thighs of the surgery bird. The ECG was calibrated to ensure a normal rhythm

and correct placement of leads. An accelerometer (HOBO UA-004, Onset Computer Corporation, Bourne, MA) was placed on the right leg of the instrumented bird and it was then placed in a 0.4 m by 0.47m by 0.3m layer cage. An accelerometer was placed on the right leg of a companion bird as well as on the right legs of the two additional birds. The use of an accelerometer to monitor poultry during depopulation was validated by Dawson *et al.* (2009) and Dawson *et al.* (2007). The companion bird was placed in the same cage with the surgery bird and the additional birds were placed in a cage adjacent to them. The layer cage facility used for this experiment was equipped with a manure belt underneath the cages. Rubber chickens were placed in the cages surrounding the birds to simulate how foam would flow around other chickens in the surrounding cages without having to increase the number of animals used in this project.

Four DSI RMC-1 PhysioTel receivers were used to record signals from the wireless transmitter. One receiver was placed on left, right, and backside of the cage of the surgery bird and the fourth receiver was placed on the top of the cage. Signals from the receivers passed through a DSI Matrix. DSI Dataquest A.R.T Acquisition software was used to monitor and record brain activity. The ECG signals were processed through BIOPAC Systems, Inc. MP30A acquisition unit and were recorded using BIOPAC Student Lab (BSL) software. Data were collected from the accelerometers using HOBO Data Logger and it was analyzed using Excel. For analysis, the X, Y, and Z acceleration channels were vector composited into one channel.

A 180 s (3 minute) baseline period was recorded to establish normal ECG and EEG patterns. Then there was a 900 s (15 minute) treatment period with a 180 s (3

minute) application time included. Data were collected from all three sensors for the duration of the trial. The data collected were analyzed to find their respective critical physiological points. ECG data was not analyzed for the purposes of this project.

CO_2 gassing was conducted in layer cages covered with thick plastic held to the side of the cage with clips to form an airtight container. Carbon dioxide gas was introduced into the chamber at a consistent rate (510 slpm) for the entirety of the 180-second application period. The gas was turned off after the finish of the application period and the birds were exposed to CO_2 for the remainder of the 900-second treatment period.

The foam was created using a compressed air foam system attached to a prototype hose and nozzle created by Dr. Morgan Farnell and his lab at Mississippi State University. The foam was applied to each cage for a period of 20 seconds. The foam filled the entire cage in this time period for all of the depopulation trials. One minute prior to the start of treatment the foam trailer was started and the foam output was observed in order to create the desired consistency. No additional foam was added to make up lost volume due to holes in the cages or bird motion.

For the CAFS with CO_2 treatment the same equipment was used to create the foam as previously described. However, for this treatment a CO_2 evaporator trailer was utilized to infuse the bubbles of foam with CO_2 gas using a manifold. The CO_2 gas application rate was 288 slpm at 580 kPa and the air application rate was 1100 slpm at 580 kPa. The foam truck and CO_2 trailer were started 1 minute prior to the start of treatment to allow foam output to be observed in order to create the desired consistency. The foam with CO_2 gas was applied to each cage for a period of 20 seconds. The foam filled the entire cages in this time period for all of the depopulation

trials. No additional foam was added to make up lost volume due to holes in the cage or bird motion.

Accelerometer data was analyzed to determine time from beginning of treatment to motion cessation. Motion cessation was defined as the point after terminal convulsions under which the acceleration sensor output reached a localized zero output.

EEG files were analyzed in DSI NeuroScore software to detect EEG silence (brain death) and unconsciousness. The recorded signal was broken down into four different regions based on an analysis using recorded time as well as EMG and EEG patterns. These include the time period before the bird received treatment (the first 180 s of the recording), the treatment period (period of time after the first 180 s to the first convulsion), the convulsion period (period of time after the first set of convulsions until the last convulsion) and the post convulsion period (the period after the last convulsion). Markers were used to label the EEG trace to match these descriptions. The raw EEG signal was analyzed in NeuroScore by marking two-second epochs in each of these areas in which there was no artifact due to movement. Artifacts are a high-amplitude spike in the EMG output (purple line) and a corresponding high-amplitude spike in the EEG trace (green line) due to movement.

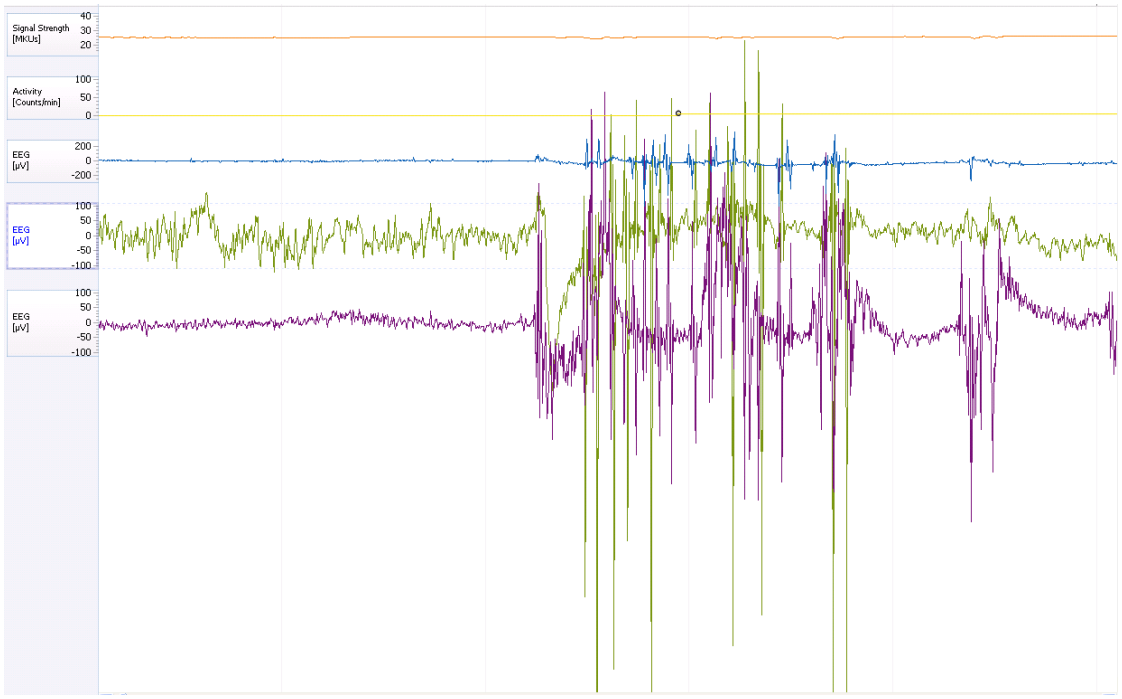

Figure 1 Representative figure of an artifact. The purple line is the EMG signal and the green line is the EEG signal indicating movement.

Epochs were labeled based on the corresponding period of time during the trial (Pre-treatment, Treatment, Convulsions and Post-Convulsions). The data was analyzed using a frequency-based analysis. Each type of wave is observed at a different frequency. Alpha is observed at 8-12 Hz, beta is at 16-24 Hz, delta is at 0.5-4 Hz, theta is at 4-8 Hz, and sigma is at 12-16 Hz. The mean EEG signal, the mean EMG signal, the values for alpha, beta, delta, theta and sigma waves, the z-ratio, and markers were exported from NeuroScore to Excel (Microsoft Corp, Redmond, WA) and charted.

To determine the point of unconsciousness in the birds the relative power band ratio alpha/delta was used. The relative power band ratio alpha/delta monitors a trend from high frequency brain wave activity to low frequency brain activity (Benson et al., 2012). The exported charts were printed and analyzed for loss of consciousness. Loss of consciousness was determined based on the location of a localized minimum after treatment application in the plotting of the alpha/delta wave. To determine the point of unconsciousness, there were four rules that were followed for an objective analysis: 1) the point of unconsciousness must occur after treatment application; 2) the loss of consciousness should occur before the convulsion phase; 3) generally, there is a rise in the signal after treatment application, believed to be a response from the birds to the treatment, then the signal begins to be suppressed; 4) when the suppression is maintained after treatment, that is the point of unconsciousness (Rankin, 2010). Analyzed data was evaluated in JMP 11.0 (SAS, Cary, NC) to determine statistical significance. Methods included Fit Y by X analysis using ANOVA and a student's t test of means. All tests were conducted at the 5% ($\alpha = 0.05$) significance level.

Chapter 3

RESULTS AND DISCUSSION

The layer hens used in this study were all single comb white leghorn layers from the University of Delaware flock, over 1 year of age. All of the birds in the study were successfully depopulated whether treated with CAFS, CAFS with CO_2, or CO_2 alone. During depopulation trials the birds were monitored with three instruments (EEG transmitter, ECG electrodes, and accelerometer). The collected data was analyzed to determine the point of unconsciousness, brain death, and terminal convulsions. The data collected from the ECG electrodes were not used for analysis in this project. All of the instruments were monitored for a total of 18 minutes, with the first 180 seconds being a baseline value used as a reference point during analysis. After the first 180-second baseline, there was a 900 second treatment period, which included a 180 second application period. The foam treatments consisted of each cage being foamed for a total of 20 seconds with additional time factored in to account for time taken to maneuver the nozzle and hose to the backside of the cages. The CO_2 gas was applied to the container with the birds for a period of 180 seconds, then the CO_2 was turned off and the birds were allowed to sit in the chamber with the CO_2 for the remainder of the treatment period.

There was not a statistically significant difference in the time to brain death between the three treatments. Carbon dioxide gassing took the longest (μ= 142. 4 sec) while CAFS (μ= 131.1 sec) and CAFS with CO_2 (μ= 135.5 sec) were more similar to each other. For this analysis, the gross signal was passed through a filter and analyzed

for the point of silence or where the mean signal over a 1 second period was steady at about 0 µV. Due to some signal irregularities, a few of the recordings were eliminated from the analysis. This is reflected in the number of replicates for the time to brain death being less than the number of birds indicated for each treatment in the material and methods section. Representative brain death traces from CAFS, CAFS w/ CO_2, and CO_2 gassing are shown in Figure 2.

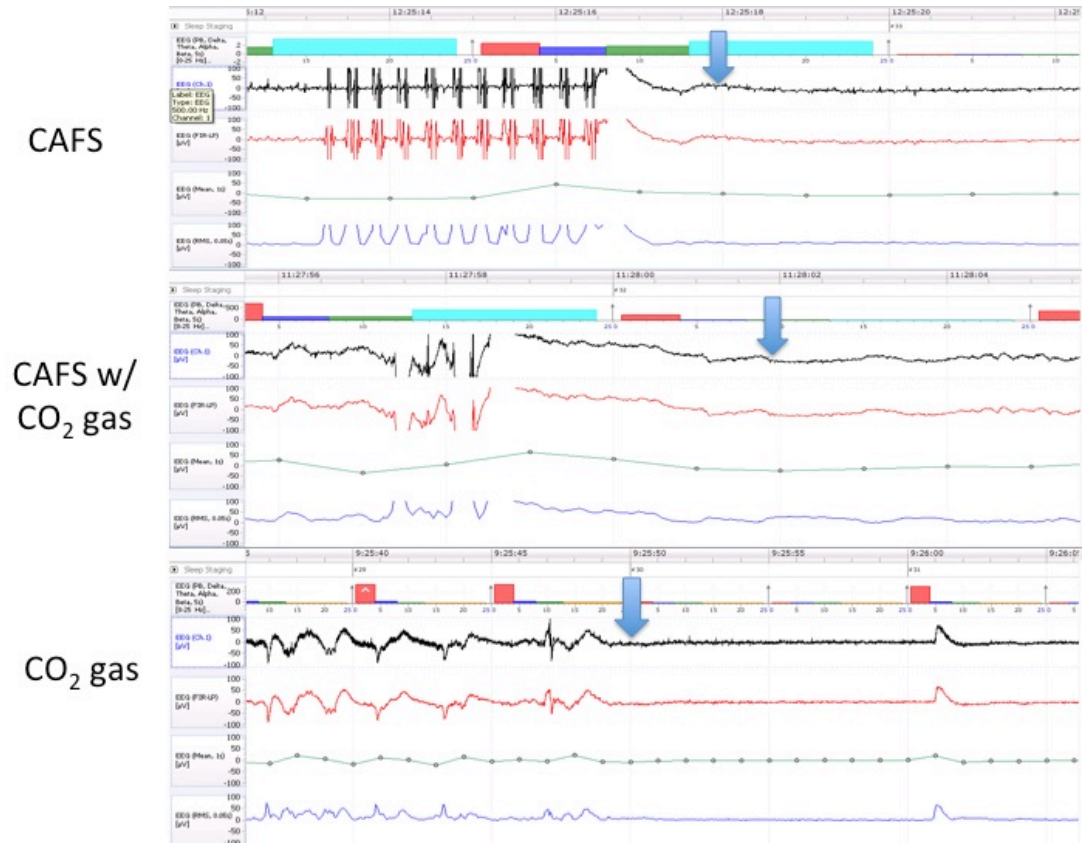

Figure 2 Filtered brain death results for CAFS, CAFS with CO_2 gas, and CO_2 gassing. The arrow indicates EEG silence (brain death).

Table 1 Comparison of the mean and standard error of brain death for the three depopulation treatments.

	Brain Death	
Treatment	Number of valid tests (n)	Time (s)
CAFS	12	131.1 ± 10.8
CAFS w/ CO_2 gas	13	135.5 ± 10.4
CO_2 gas	14	142.4 ± 10.0

The time to terminal convulsions, or motion cessation, of the birds was determined through the use of an accelerometer. The results show that there was no statistically significant difference between CAFS (μ= 211.4 sec), CAFS with CO_2 gas (μ= 224.0 sec), and CO_2 gas (μ= 226.4 sec). These data were used to determine when the bird enters and concludes the terminal convulsion phase of death. The terminal convulsion phase is an unalterable point, where the bird is no longer conscious. The verification of the use and comparison of accelerometer data to ECG and EEG data was shown in Dawson et al. 2007 and Dawson et al. 2009. These two studies showed that motion cessation can be used to establish the timing of the completion of the convulsion phase and as an estimator of the time to brain death (Dawson et al. 2007; Dawson et al. 2009).

Table 2 Comparison of the mean and standard error for time to motion cessation for the three depopulation treatments.

	Motion Cessation	
Treatment	Number of Valid Tests (n)	Time (s)
CAFS	15	211.4 ± 14.8
CAFS w/ CO2 gas	14	224.0 ± 15.3
CO2 gas	14	226.4 ± 15.3

The time to unconsciousness was evaluated using the EEG signals recorded from the wireless transmitter that was surgically implanted into the bird. From a welfare point of view the time it takes to reach unconsciousness is significant because it shows the point at which the bird is no longer cognizant of its surrounds or feeling any pain. The results from the analyzed data show that there was a significantly difference between CO_2 gas (μ= 38.5 sec) and the foam treatments. There was no statistically significant difference between CAFS (μ= 19.5 sec) and CAFS with CO_2 gas (μ=16.9 sec). Due to some signal irregularities and interference, some of the recordings were eliminated from the analysis. This is reflected in the number of replicates for the time to unconsciousness being less than the number of birds indicated for each treatment in the material and methods section. Illustrative unconsciousness charts for all three treatments are shown in Figures 3, 4 and 5.

Table 3 Comparison of the mean and standard error for time to unconsciousness for the three depopulation treatments.

	Unconsciousness	
Treatment	Number of Valid Tests (n)	Time (s)
CAFS	8	19.5 ± 4.3
CAFS w/ CO2 gas	12	16.9 ± 3.5
CO2 gas	11	38.5 ± 3.7

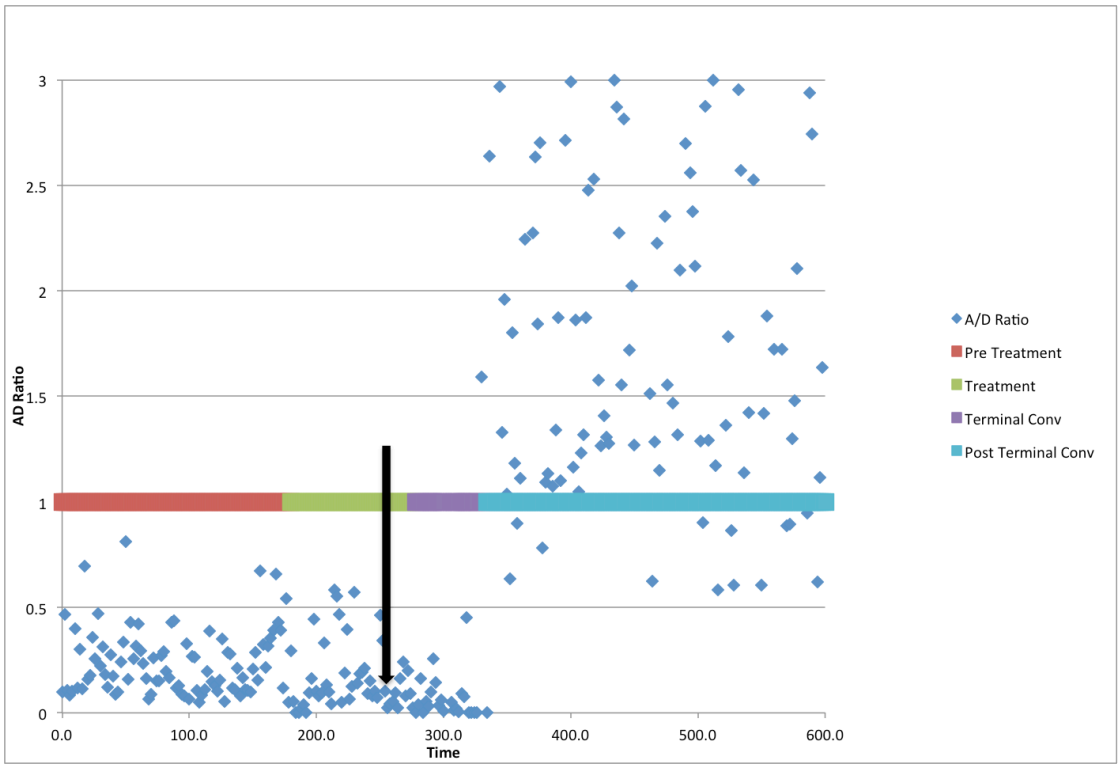

Figure 3 Charting of the A/D ratio based on markers placed in the raw signal. Point of unconsciousness is shown as the first localized minimum after treatment application. Layer 35 from 7/31/14. Bird became unconscious at 256 seconds (76 seconds after application of the CO_2 treatment), as indicated by the arrow.

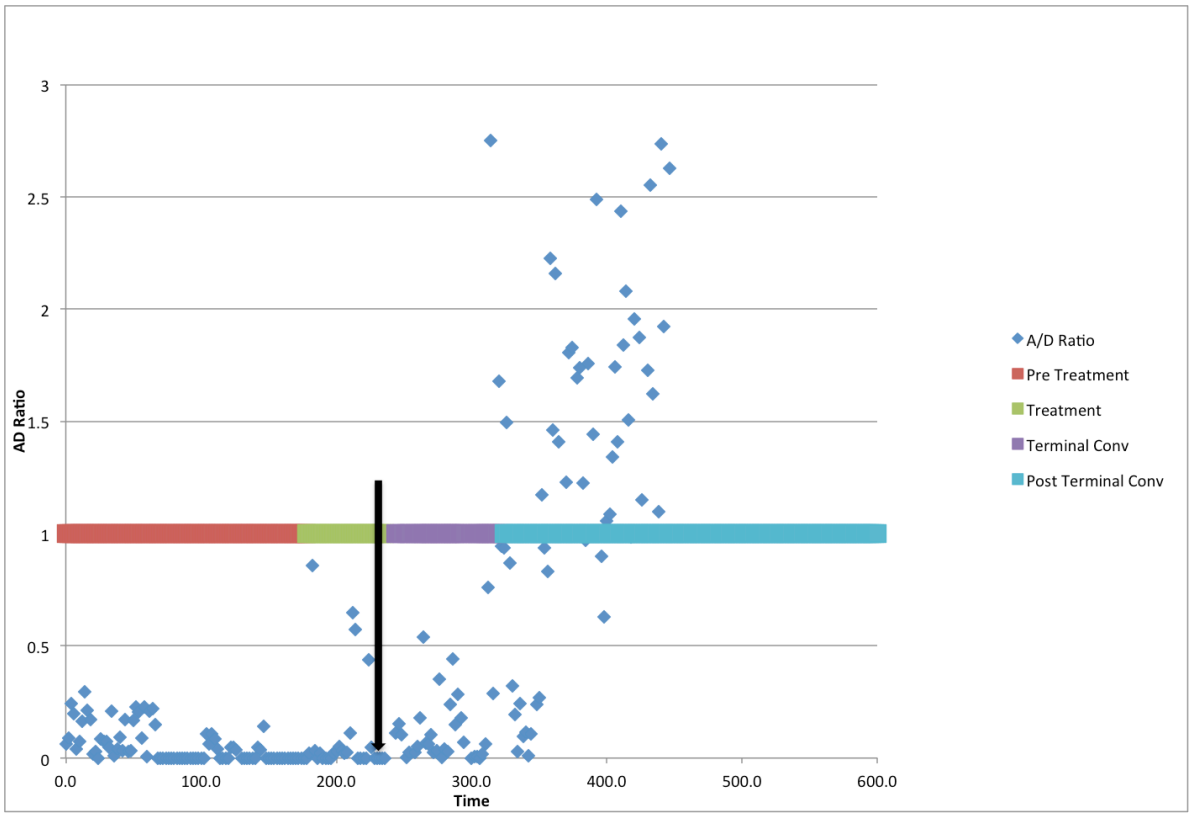

Figure 4 Charting of the A/D ratio based on markers placed in the raw signal. Point of unconsciousness is shown as the first localized minimum after treatment application. Layer 11 from 6/24/2014. Bird became unconscious at 226 seconds (26 seconds after application of the CAFS treatment), as indicated by the arrow.

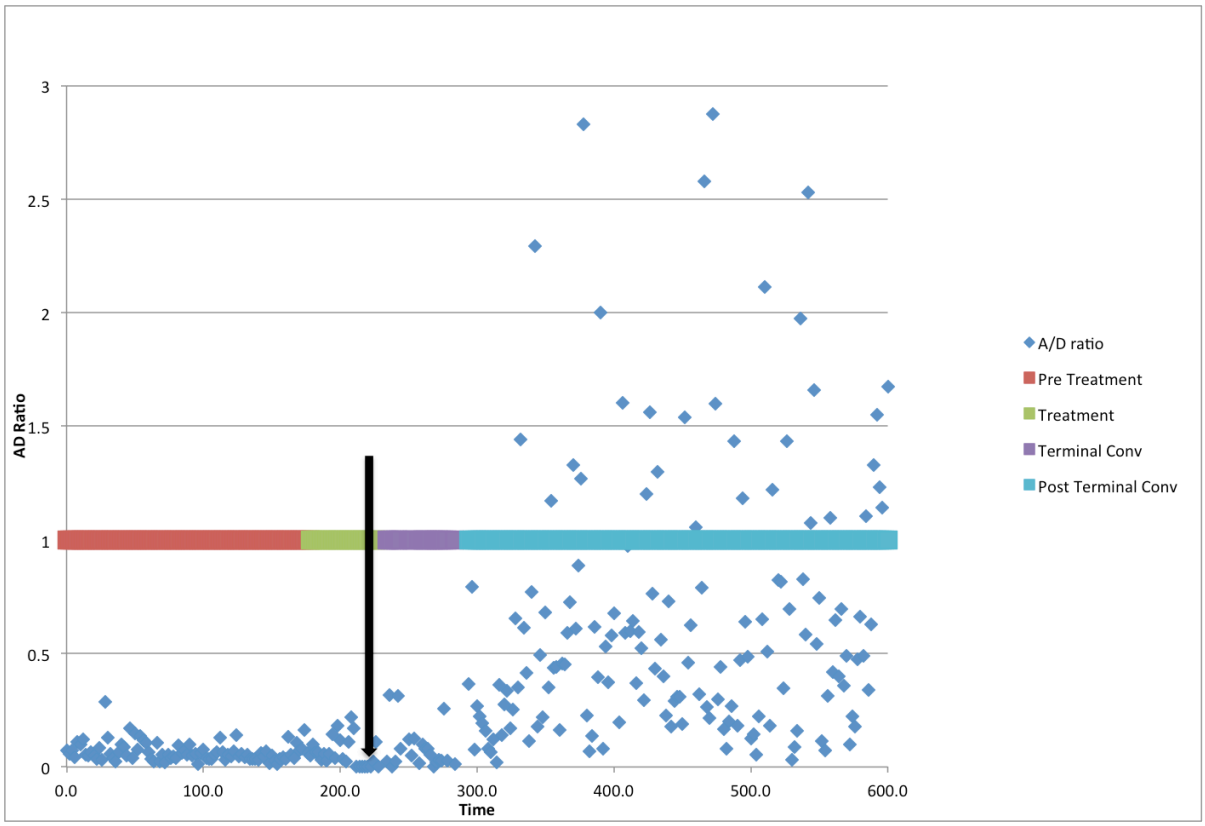

Figure 5 Charting of the A/D ratio based on markers placed in the raw signal. Point of unconsciousness is shown as the first localized minimum after treatment application. Layer 44 from 8/7/2014. Bird became unconscious at 214 seconds (14 seconds after application of the CAFS with CO2 gas treatment), as indicated by the arrow.

The results of this experiment show that CAFS with or without CO_2 gas is more effective at causing unconsciousness than CO_2 gas. However, there is no statistical significance between the CAFS and CAFS with CO_2 gas treatments. The CAFS and CAFS with CO_2 treatments also resulted in faster times to brain death and terminal convulsions. However, these data were not statistically significant. Both CAFS and CAFS with CO_2 gas were more consistent for times to unconsciousness, with less variation from the mean compared to CO_2 gassing. Conversely, the CAFS treatment was less consistent when considering time to brain death, with more variation from the mean compared to CO_2 and CAFS with CO_2. For time to motion cessation, CO_2 gassing had the most consistent times, with less variation from the mean compared to CAFS and CAFS with CO_2. Overall, the results of the experiment show that CAFS can be used to successfully depopulate caged layer hens. The addition of CO_2 gas to CAFS, however, does not make a difference in the effectiveness of the foam in depopulating the layer hens.

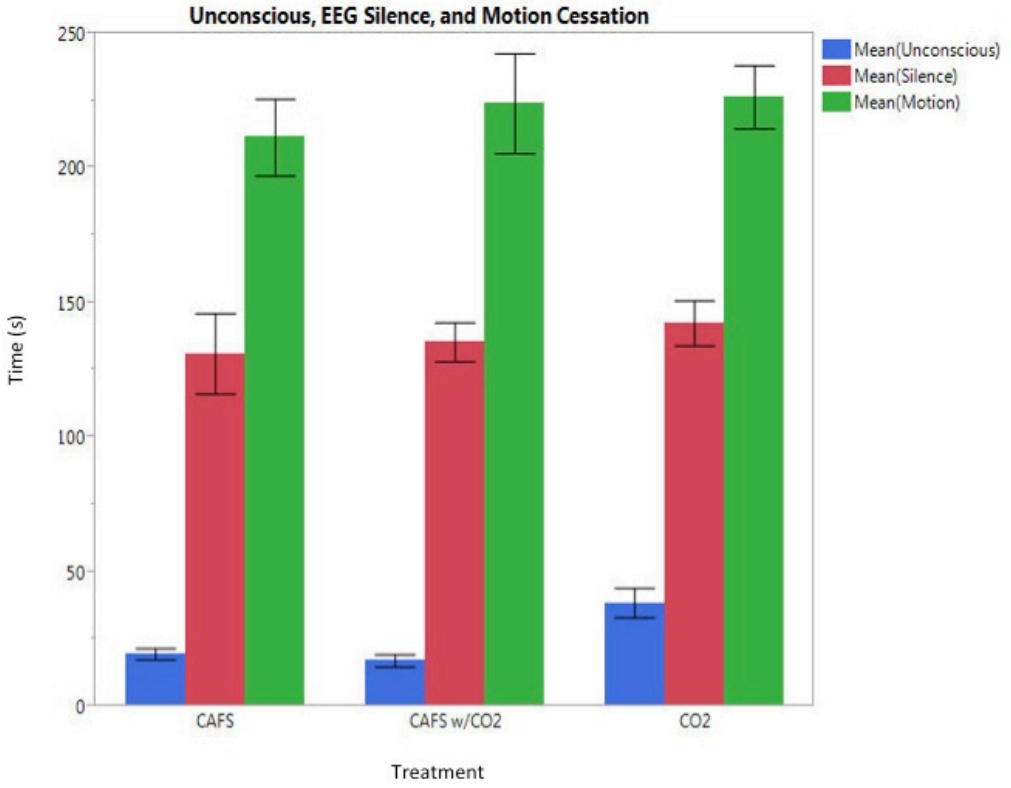

Figure 6 Summary chart of the physiological parameters evaluated in this study. Time to unconsciousness was the only parameter with a statistically significant difference between the three treatments.

Chapter 4

CONCLUSIONS

Emergency depopulation is not a glamorous part of working in the poultry industry and it is something everyone hopes is never necessary. However, as exemplified by the current outbreak of avian influenza in the Midwestern United States, it is clear that the chance of poultry industry workers being involved in depopulation is very high. In cases where depopulation is deemed necessary, the responders need to be able to handle the situation in the most effective way that is also the safest for the responders. The current methods of depopulation that are approved for poultry are not as effective for caged layer hens as they are for other poultry. This is due the fact that layer hens are kept in raised cages unlike the majority of other poultry, which are floor reared. The most common methods of depopulation currently used for caged layer hens include CO_2 gassing and cervical dislocation. Cervical dislocation is very time consuming, especially in a large layer facility, which can have thousands of birds. Cervical dislocation also requires the responders to be in contact with the birds for a longer amount of time, which is dangerous when it comes to disease outbreaks as it can lead to spread of the virus, or in the case of H5N1, which may actually spread to humans.

Carbon dioxide gassing, while effective, also has some serious drawbacks that need to be considered when depopulating caged layer hens. When gassing layer hens the container that they are in needs to be airtight so as to prevent the escape of CO_2 gas and to allow the gas to reach a lethal level. This can be difficult and time consuming if the laying facility is large or has experienced any structural damage due

to old age or natural disaster. Another disadvantage to CO_2 gassing is that it is toxic to humans in addition to being toxic to poultry. Therefore, responders who are dealing with CO_2 gas must wear a self-contained breathing apparatus and appropriate gas concentration meters.

As mentioned in the literature review, the use of water-based foam has been conditionally approved by USDA-APHIS. The drawback is that it has not been approved for use in non-floor reared poultry such as caged layer hens. Unfortunately, the cages represent a barrier for foam depopulation. Foam needs to be able to penetrate the cage to affect the birds, but have a sufficient residency time for depopulation to occur in the cage. The current generation of foam depopulation equipment, including the Kifco Avi-Guard and nozzle-based systems including the Spumifer nozzle do not create foam that is capable of providing an appropriate balance between residency time and cage penetration.

In general, foam is a safer option for responders and is suitable for structurally damaged houses but it is not toxic to humans and poses no threat to the responders. The CAFS with CO_2 gas showed no improvements to the regular CAFS. Adding CO2 gas to the foam is more costly and also makes the depopulation process require more materials. As such, there is no benefit of adding CO_2 to CAFS.

This study has shown that a compressed air foam system is a valid option for depopulation of caged layer hens, provided that the facility contains cages with a manure belt underneath. This information allows responders to disease outbreaks or natural disasters to have another option when deciding how to depopulate layer hens. The data in this project will hopefully assist organizations such as the AVMA and

USDA in evaluating a compressed air foam system as an appropriate depopulation method for caged layer hens.

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