

Evaluation of Ventilation Shutdown in a Multi-level Caged System

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Primary Audience: Flock Supervisors, Poultry Veterinarians, State Emergency Service Personnel

SUMMARY

In 2015, the United States experienced an extensive outbreak of highly pathogenic avian influenza resulting in the depopulation of 42.1 million commercial egg-laying hens. Existing mass depopulation methods in multilevel caged systems utilized throughout the egg industry provide a challenge for producers and emergency personnel. An alternative depopulation method with potential for use in the egg industry is ventilation shutdown (VSD), which uses a combination of increasing heat and carbon dioxide (CO₂) levels to promote death by hyperthermia and hypoxia. This proof-of concept study was designed to evaluate the effectiveness of VSD, VSD with supplemental heat (VSDH), and VSD with CO₂ (VSDCO₂) as alternative mass depopulation methods in a multilevel caged system. Assessment parameters included ambient and core body temperatures, time to death, and survivability. Time to death for VSD, VSDH, and VSDCO₂ were 3.75, 2, and 1.5 h, respectively. Survivability in VSD did not meet the flock depopulation standard of 100% lethality. Based on 100% lethality and time to death, VSDH and VSDCO₂ are equivalent in their ability to effectively depopulate hens in a multi-level caged system. Air mixing should be used to prevent stratification of heat or CO₂ concentrations between the top and bottom levels of a caged system. While the data emulate a commercial layer environment, application of the techniques in this study still need to be evaluated in a commercial setting.

Key words: ventilation shutdown, heat, CO₂, mass depopulation, multi-level caged systems, laying hens

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DESCRIPTION OF PROBLEM

In 2015, the United States experienced an extensive outbreak of highly pathogenic avian

influenza in commercial and backyard poultry. The outbreak involved 3 of the 4 US flyways spreading initially through migrating waterfowl, resulting in the destruction of 49.6 million birds; 42.1 million were commercial egg-laying hens and pullets [1]. Timely depopulation was

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identified as a critical measure needed to contain the outbreak and lack of timely depopulation was suspected to have contributed to the further spread of the disease [2]. The United States Department of Agriculture (**USDA**) recognizes mass depopulation as “a method by which large numbers of animals must be destroyed quickly and efficiently with as much consideration given to the welfare of the animals as practicable [3].” Existing emergency mass depopulation methods, including carbon dioxide (CO₂) kill carts, CO₂ injection of entire production houses, and water-based expanding foam, were not able to be performed in a timely manner due to significant lack of resources. This created a situation that prolonged suffering of infected birds and increased the biosecurity risk.

The multi-level caged systems utilized throughout most of the egg industry provide a challenge for producers and emergency personnel in the face of emergency disease situations. The primary emergency depopulation methods for egg layers—CO₂ kill carts and CO₂ injection—require 1) extensive human–bird interaction while the birds are in an infective state, 2) on-site resources that can be limited when faced with the potential of depopulating multiple farms with over a million birds, and 3) increased safety risks for workers when using CO₂ injection [4]. The alternative method of water-based foam, while useful in floor systems, has not been shown to be effective in multi-level caged systems. The American Veterinary Medical Association and American Association of Avian Pathologists have indicated the need for timely, definitive, and decisive alternative emergency depopulation methods in caged layers. Alternative depopulation methods are to be used if other primary methods cannot be implemented or resources are not available within the expected time frame for completion of the depopulation [3]. An alternative emergency depopulation method with potential for use in the egg industry is ventilation shutdown (**VSD**). Ventilation shutdown uses a combination of naturally and/or artificially increasing heat and CO₂ levels within a sealed poultry house to promote death by hyperthermia (extreme heat) and hypoxia (low oxygen levels). Hyperthermia is induced by achieving ambient temperatures (T_a) that exceed the hen’s core body temperature (T_b).

To ensure VSD is effective, it is suggested that the T_a within the poultry house rises to 104°F (40°C) or greater [5]. This quick rise in T_a can be attained by sealing the air inlets and turning off ventilation fans [3]. Death is achieved by reaching the hen’s upper lethal T_b of 113°F (45°C) [6].

While ventilation failure has occurred in the egg industry by accident through loss of electricity [7, 8], the physiological effect of VSD on commercial laying hens is still unclear. The following proof-of-concept study was designed to evaluate the effectiveness of VSD, alone and with supplemental heat or CO₂, in a multi-level caged system.

MATERIALS AND METHODS

Animal Care

The project was approved by North Carolina State University’s Institutional Animal Care and Use Committee under ID# 15–125-A. All animals were maintained on North Carolina State University property for the duration of the study. The study was monitored by animal welfare specialists and veterinarians employed by the university.

Prior to the project, North Carolina State University’s Department of Environmental Health and Safety (NCSU-EHS) was contacted to ensure proper training and supervision for all experiments that included exposure to potentially hazardous levels of CO₂. North Carolina State University personnel underwent a Qualitative Fit test and were compliant with the training requirements to use Self-Contained Breathing Apparatus (**SCBA**) equipment for entering the room during experiments where CO₂ would be higher than 1,000 ppm, as prescribed by OSHA Standard 29 CFR 1910.134 [9].

Experimental Animals

At the start of each experiment, 144 white laying hens ranging in age from 68 to 80 wk were obtained from a commercial egg-laying company. Hens were transported to the Poultry Entomology Research Unit (**PERU**) at North Carolina State University in poultry transport

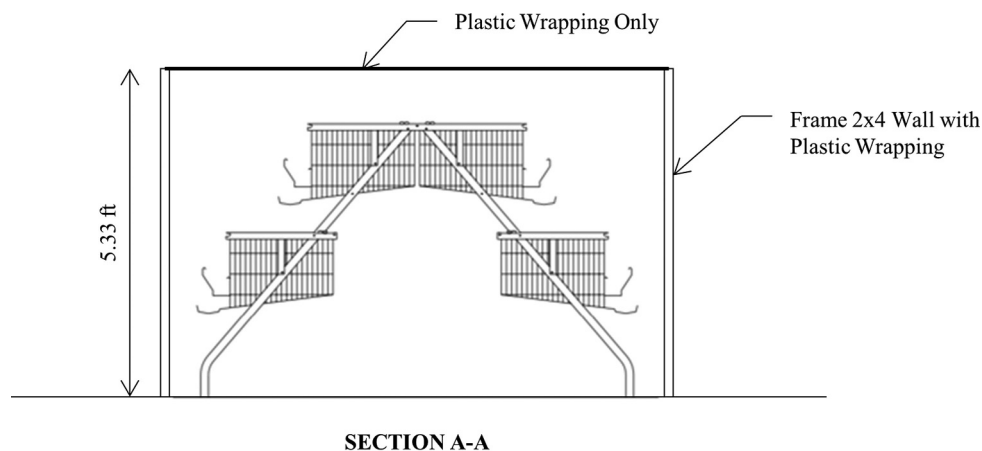


Figure 1. Multi-level full stair step conventional caged system with 7.5 ft × 15 ft inner chamber built for experiments 1–3.

coops and housed in a conventional multi-level, full stair step caged system as seen in Figure 1 for 5–7 d to acclimate to the system prior to treatment application. Three hens were placed in each cage upon arrival in accordance with the “Guide for the Care and Use of Agricultural Animals in Research and Teaching” [10]. Each cage was 12 in. × 18 in. (72 in.² per bird) and provided 12 in. feeder space and 2 nipple drinkers. Feed and water were provided ad libitum.

Experimental Room Design

The multi-level caged systems were kept in a 29.5 ft × 15 ft × 8 ft room at the PERU (Figure 2). On the south end of the room, an 8 ft × 7 ft roll door was flanked by one 12 in. fan [11] and one 24 in. fan [11]. Since Universal Cooperatives is no longer in operation and no fan curves were retained, the airflow rates of the fans are not known; however, 12 in. and 24 in. ventilation fans have airflow rates of 850–1,860 cfm and 5,500–7,200 cfm, respectively, at 0.05 in. of static pressure [12]. The north end contained a set of 5.83 ft × 6.5 ft glass panel doors and an intake vent. A 60,000 BTU propane heater [13] was in the northeast corner. A partition was built to allow for evacuation of potentially dangerous levels of CO₂ as requested by NCSU-EHS. This reduced the room’s dimensions to 19.5 ft × 15 ft × 8 ft; a reduction of 1,200 ft³ from the original room size and provided hens with 16.3 ft³. The

partition was framed with 2 × 4 studs and 2 in. polystyrene. A 58 in. × 25 in. evacuation panel was cut in the center of the partition to allow for the quick release of gases to the outside of the building. An inner chamber around the caged system was developed to decrease the headspace volume per bird from 16.3 ft³ to 4.1 ft³ to approximate industry standards [14]. The 15 ft × 7.5 ft × 5.3 ft chamber was constructed of 2 × 4 studs covered with 10 mil polyethylene plastic (Figure 2). The walls of the inner chamber attached to the false wall completely enclosing the caged system on each side. The chamber was sealed when a 10-mil polyethylene plastic ceiling was screwed into place. To seal the room, the ventilation was turned off and the seams of the partition, evacuation panel, and intake vent were taped with a 1/2 in. foam adhesive to prevent any airflow or leakage. The drinking water system was turned off and drained. The glass panel doors were then shut and sealed with 2 in. adhesive around the entire door frame. The 60,000 BTU propane heater, located outside the inner chamber, was utilized to equalize the T_a inside the inner chamber with the T_a in the room. This was done to mimic the heat transfer that would occur in a heavily insulated conventional cage facility.

Environmental and Animal Monitoring

Ambient temperature and relative humidity (RH) probes [15] were placed on each level

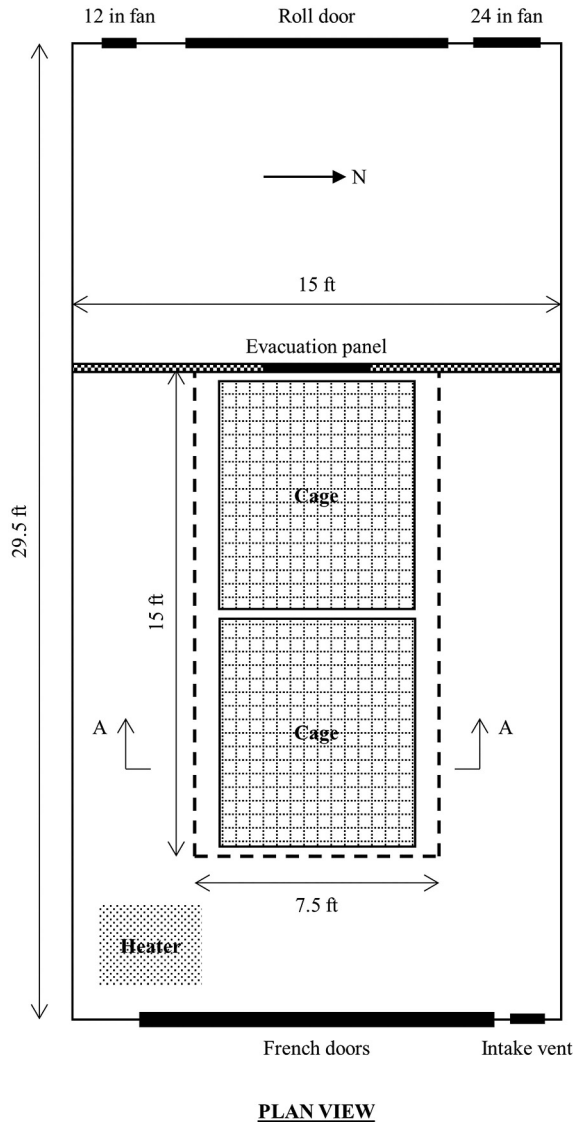


Figure 2. Plan view of the 29.5 ft × 15 ft × 8 ft room at the Poultry Entomology Research Unit at North Carolina State University. The 7.5 ft × 15 ft inner chamber, as seen in dotted lines, was used in experiments 1–3.

of the caged system, within <1.3 ft vertically of one another, as well as in the back and front of the room to collect environmental data. Outside environmental conditions were obtained via The National Weather Service [16]. The intake pumps for two 100% and two 5% CO₂ sampling data loggers [17] were set up the same as the T_a and RH probes. Moisture traps were added to the sampling lines to prevent sensor operation being impeded by condensation. The data loggers were placed by the glass panel doors so that room con-

ditions could be monitored from outside the room. Ambient temperature, RH, and CO₂ concentrations were recorded every minute throughout each experiment.

Four cameras [18] were positioned on the cages to allow for visual monitoring of the hens to determine loss of posture, as described by Webster and Fletcher [4], and discourage entry into the room until all birds were observed to be deceased. Body weights and core body temperatures (T_b) were taken from 15 randomly selected hens prior to sealing the room. Core body

temperatures were also taken from 10 randomly selected hens after time of death (TOD) was visually determined and the room was opened. All T_b were taken via a cloacal temperature probe [19].

The following experiments were conducted from June through September in Raleigh, North Carolina. A 7 h time limit was set for all data collection.

Experiments

Experiment 1: Ventilation Shutdown In this experiment, the ventilation system was shut down and the chamber and room sealed as described above. No supplemental CO₂ or heat was added to the inner chamber.

Experiment 2: Ventilation Shutdown With Supplemental Heat In experiment 2, the ventilation system was shut down and room sealed in the same manner as experiment 1. Supplemental heat was added to the inner chamber. Prior to sealing the inner chamber and room, two 1.5 kW electric heaters [20] were placed at opposite ends of the inner chamber. The 60,000 BTU propane heater and electric heaters were turned on to begin increasing the temperature to achieve a T_a of 104–105.8°F (40–41°C).

Experiment 3: Ventilation Shutdown With CO₂ In experiment 3, the ventilation system was shut down and room sealed in the same manner as experiment 1. Supplemental CO₂ was injected into the inner chamber. Prior to sealing the inner chamber and room, CO₂ distribution hoses were positioned within the inner chamber on the opposite side of the partition so that the room would fill with CO₂ from one end. The CO₂ cylinders were located outside the sealed room and to the west of the evacuation panel. The roll door was open as required by NCSU-EHS. A 1,500-W heat gun [21] was used to warm the regulators to ensure controlled release of CO₂ through two $\frac{1}{4}$ in. diameter clear polyvinyl chloride (PVC) tubes [22] fed into the inner chamber at floor level. A small 100 cfm fan [23] was installed in the partition to evacuate air from the room as the CO₂ displaced the existing air to ensure that the room did not get overpressurized. Two 100% CO₂ sensors were used, with the sampling inlet of one located on top of the lower cage and the sampling inlet of the other located

Table 1. The Start and End Time for Ventilation Shutdown (VSD), Ventilation Shutdown With Supplemental Heat (VSDH), and Ventilation Shutdown With CO₂ (VSDCO₂).

Experiment	Start time	End time	Total duration (h)
VSD	08:30	12:15	3.75
VSDH	08:00	10:00	2
VSDCO ₂	08:45	10:00	1.5

on top of the upper cage, with both sampling lines located about midway along the chamber. For the first 9 min of shutdown, 1 CO₂ sensor was used to monitor CO₂ concentration from the exhaust of the 100 cfm fan. After 9 min, the CO₂ sensor's sampling inlet was switched from the fan outlet to the sampling location within the inner chamber. North Carolina State University SCBA trained personnel were on premise in the event entry into the room was necessary. The CO₂ regulators were turned on to supply ~0.65 lb CO₂/min and left until concentrations in the room reached 40% at the top level of the multi-level caged system [24, 25].

RESULTS AND DISCUSSION

Table 1 provides the length of time each experiment was conducted. University veterinarians were onsite throughout the duration of all experiments to visually determine TOD. Table 2 demonstrates the average hen and environmental parameters pre- and post-ventilation shutdown for VSD, VSD with supplemental heat (VSDH), and VSD with CO₂ (VSDCO₂) experiments. Ambient temperature, RH, and CO₂ readings were compiled for each minute throughout each experiment.

Ventilation Shutdown

The temperature increase in VSD was rapid and the maximum inner chamber T_a achieved was 102.2°F (39°C). The inner chamber T_a increased from 73.6°F (23.1°C) to 102.2°F (39°C) in <2.5 h and then stayed constant for 30 min before decreasing to 98.2°F (36.8°C) at the end of the experiment (Figure 3a). The temperature achieved did not meet the DEFRA recommended guidelines of 104°F (40°C) for VSD [5]. Average outside T_a was 75.5°F (24.2°C) and average outside RH was 73% [16]. Relative humidity increased rapidly from 74 to 87% within

Table 2. Average Hen and Environmental Parameters Pre- and Post-Ventilation Shutdown During Ventilation Shutdown (VSD), Ventilation Shutdown With Supplemental Heat (VSDH), and Ventilation Shutdown With CO₂ (VSDCO₂).

Treatment	Pre-shutdown				
	Body weight (lbs)	Core body temperature °F (°C)	Volume (ft ³ /hen)	Inner chamber T _a °F (°C)	Inner chamber RH (%)
VSD	3.327	105.8 (41.0)	4.1	73.6 (23.1)	74.0
VSDH	3.609	105.1 (40.6)	4.1	74.3 (23.5)	82.8
VSDCO ₂	3.774	105.4 (40.8)	4.1	78.4 (25.8)	84.9
Treatment	Post-shutdown				
	Core body temperature °F (°C)	Inner chamber T _a °F (°C)	Inner chamber RH (%)	Inner chamber CO ₂ (%)	Survivor (%)
VSD	111.7 (44.3) ¹ 109.3 (42.9) ²	98.2 (36.8)	90.0	3.7	2.8
VSDH	112.3 (44.6) ²	111.2(44.0)	73.0	1.9	0
VSDCO ₂	99.4 (37.4) ²	83.3 (28.5)	88.9	40.8	0

¹Core body temperature of surviving hens.

²Core body temperature of dead hens.

35 min. The concurrent rise in T_a and RH in the inner chamber, along with the removal of water access by draining the drinking water system, contributed to the hen's inability to dissipate body heat through evaporative cooling. Like T_a, RH within the inner chamber rose to a maximum of 92% before declining slowly as mortality increased. Carbon dioxide concentrations increased linearly and peaked at 3.7% (Figure 3b). Carbon dioxide concentrations had a similar trend to temperature by peaking for about 30 min before declining. The decline in the inner chamber T_a and CO₂ near the end of shutdown was attributed to the decreased heat produced as mortality increased. The inability to reach temperatures at or higher than 104°F (40°C) and the loss of heat production are disadvantages to VSD alone and could be a problem in large-scale depopulation during cooler seasons.

For VSD, 2.8% of the hens survived. While the inner chamber T_a only reached 102.2°F (39°C), T_b steadily increased, reaching temperatures as high as 111.7°F (44.3°C) for surviving hens and 109.3°F (42.9°C) for dead hens. Core body temperatures were taken within 2 min after the room was opened for entry. Although the survival rate was low, it did not meet 100% lethality for flock depopulation [26].

VSD With Supplemental Heat

The addition of heat for VSDH resulted in the inner chamber T_a increasing from 74.3°F

(23.5°C) to a maximum of 113°F (45°C) in <1 h (Figure 4a). Average outside T_a was 78°F (25.6°C) while average RH was 75% during the experiment [16]. In the latter half of the 2-h experiment, the inner chamber T_a slowly declined ending at 111.2°F (44.0°C) (Figure 4a). Relative humidity in the inner chamber increased to 84% before declining rapidly to 73% at the end of depopulation.

Carbon dioxide sensors in VSDH were not available for the first 8 min of the experiment due to equipment malfunction. Average CO₂ concentration in the inner chamber increased linearly from ~0.65% to a maximum of 2% in 1 h and then declined slowly to 1.9% by the end of the experiment (Figure 4b). As the unvented propane heater used up oxygen in the sealed room, the heater shut down due to shortage of oxygen; hence, desirable temperature rise may be difficult to maintain if unvented heaters are used for VSDH. Changes in T_a and CO₂ concentrations (Figure 4) were similar to the changes observed in VSD (Figure 3). Gradual decline in inner chamber T_a, RH, and CO₂ concentrations in the latter half of the study could be attributed to reduced heat and CO₂ production from hyperthermic birds. Due to heat rising within the inner chamber, the temperature initially stratified with the hens in the top level dying before the hens in the bottom level of the caged system. In commercial multi-level caged systems, where the separation could be >6.56 ft between the top and bottom levels, stratification of

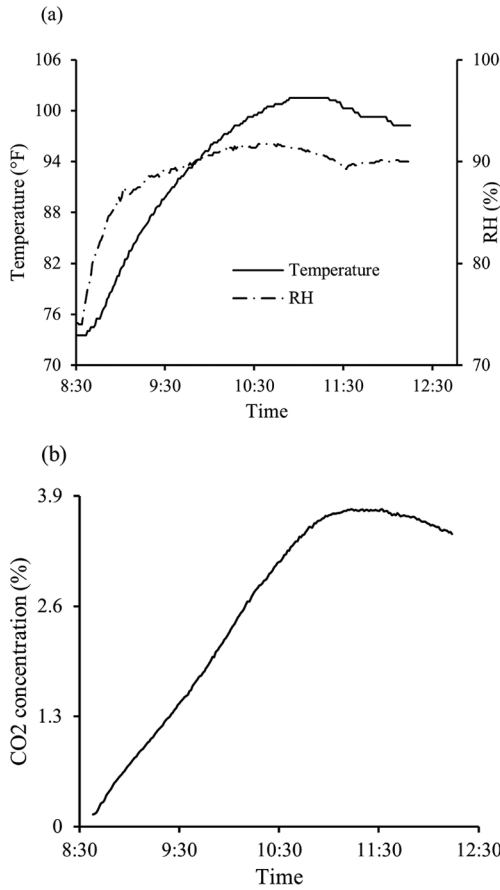


Figure 3. Changes in the inner chamber (a) T_a and RH and (b) CO_2 concentrations during ventilation shutdown (VSD) (08:30–12:15). Environmental parameters were measured every minute and their trends are based on the average of 4 sensors except for CO_2 which is based on 4 sensors from 08:00 to 11:18 and 3 sensors thereafter.

temperature and, therefore, time to death could be a concern for the laying hens on the bottom levels.

Ventilation shutdown with supplemental heat was effective in meeting the intent of flock depopulation standards of 100% lethality. The added heat resulted in an average T_b of 112.3°F (44.6°C). The rapid rise in T_b resulted in hyperthermia without the large drop in environmental temperatures, eliminating the possibility of survivors as seen in VSD alone.

VSD With CO_2

Due to the nature of VSDCO₂, hypoxia by CO_2 was likely the main cause for mortality.

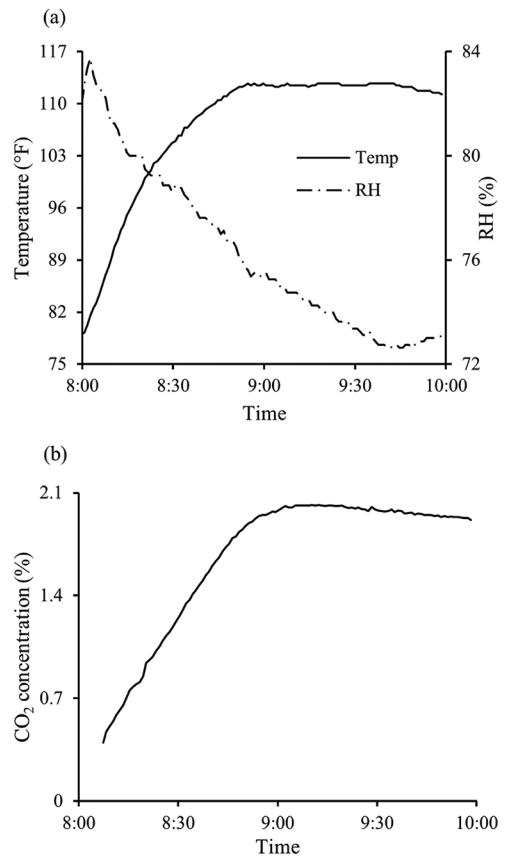


Figure 4. Changes in the inner chamber (a) T_a and RH and (b) CO_2 concentrations during ventilation shutdown with supplemental heat (VSDH) (08:00–10:00). Environmental parameters were measured every minute and their trends are based on the average of 4 sensors.

Ventilation shutdown with CO_2 was successful in producing 100% lethality. Core body temperatures were taken within 5 min after the room was opened for entry and CO_2 evacuated. The average T_b of birds in VSDCO₂ was 99.4°F (37.4°C), much lower than T_b seen in VSD and VSDH. The inner chamber T_a increased from 78.4°F (25.8°C) to 83.3°F (28.5°C) in <45 min and declined gradually thereafter, whereas RH declined from 85% at the start of study to 80% at the end (Figure 5). Average outside T_a was 82.5°F (28.1°C) while average RH was 73% during the experiment [16].

Data from the 5% CO_2 sensors used to measure CO_2 concentrations in the small exhaust fan were discarded for the first 9 min after adding supplemental CO_2 to the room. Carbon

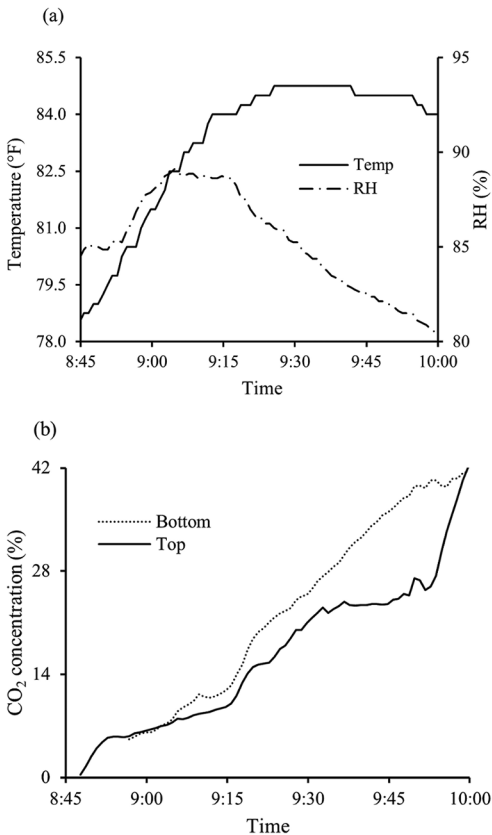


Figure 5. Changes in the inner chamber (a) T_a and RH and (b) CO_2 concentrations during ventilation shutdown with CO_2 (VSDCO₂) (08:45–10:00). Environmental parameters were measured every minute and their trends are based on the average of 4 sensors for T_a and RH and 2 sensors for CO_2 (1 bottom level, 1 top level).

dioxide concentrations in the room increased rapidly and linearly to 41% by the end of the experiment (Figure 5b). As shown in Figure 5b, the top level CO_2 sampling data logger located <1.3 ft above the bottom level CO_2 sampling data logger recorded lower CO_2 concentrations due to the time it took for the inner chamber to fill up with CO_2 and the weight of CO_2 compared to O_2 . Like VSDH, mortality in VSDCO₂ was stratified based on location of the hen within the caged system. The lower level cage succumbed to the CO_2 first followed by the upper level cage. The time it takes to for hypoxic concentrations of CO_2 to reach the top level in a commercial multi-level caged system could differ in the time to death for the hens on the bottom versus top level.

CONCLUSIONS AND APPLICATIONS

1. Survivability in VSD does not meet the flock depopulation standard of 100% lethality.
2. Based on 100% lethality and time to death, VSDH and VSDCO₂ proved equivalent in their ability as an effective alternative mass depopulation method.
3. Air mixing should be used with VSDH and VSDCO₂ to prevent stratification of heat or CO_2 concentrations between the top and bottom levels of a multi-level caged system.
4. Ventilation shutdown with the addition of supplemental materials like heat and CO_2 appears to be a more effective way to depopulate a laying hen flock than exposure to VSD alone in a multi-level caged system.
5. Ventilation shutdown with supplemental heat and VSDCO₂ should be considered alternative emergency mass depopulation methods and used only if other acceptable methods cannot be implemented or resources are not available within the expected time frame for completion of the depopulation.
6. The data emulate a commercial layer environment; however, application of the methods in this study still need to be evaluated in a commercial setting.

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