# IMPROVING CURRENT MASS DEPOPULATION METHODS FOR AN EMERGENCY RESPONSE IN A CAGE-FREE HOUSING SYSTEM

By

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## ABSTRACT

Table egg producers are switching to cage-free production systems as state legislation is passed requiring removal of conventional systems. Utilization of AVMA preferred depopulation methods may not always be feasible in cage-free systems due to complicated structure of building, size, resource availability, and concerns for worker safety. Additionally, carcass removal after mass depopulation in cage-free systems may be challenging due to onset of rigor mortis as hens have been observed grasping onto metal structure with feet. Two experiments were conducted to improve current depopulation response in cage-free systems. Approximately 1,800 Lohmann Brown hens aged 56 weeks were housed in cage-free aviaries (Big Dutchman Natura 60) in 4 rooms (same hens and system were used in both experiments). In experiment 1, a combination of UV flash (1 pulse) and darkening of floor lights was examined on laying hens to elicit a movement response, particularly moving out of aviary system and onto floor area. Four treatments were as followed: 1) control, 2) UV lights flashed for 10-sec (UV), 3) floor lights turned off and UV lights flashed for 10-sec (DF+UV), and 4) floor lights turned off (DF). Results indicated that when UV flash was combined with darkened floors (DF+UV) in AM application, a greater difference in number of hens ($P \leq 0.05$) was observed when compared to other lighting treatments; while in PM application, this difference was only apparent when comparing DF+UV to the control. UV light flash influenced hens' behavior, with more stress related behaviors (standing alert) apparent in both UV treatments, while more hens exhibited normal behaviors (preening and wing flapping) in non-UV light treatments. Although hens moved to desired location (floor area) after usage of UV light flash, technique may not be effective for long term movement since hens moved back into aviary within 6-min. In experiment 2, steam was assessed as a "plus" for VSD+ depopulation. Four VSD+ treatments were as follows: 1) VSD+ heat to 40°C (VSD-H), 2) VSD with steam to 40°C (VSD-S), 3) VSD with heat to 40°C and then steam to maintain temperature (VSD-HS), and 4) VSD with steam to 40°C and then heat to maintain temperature (VSD-SH). Treatments that utilized steam caused laying hens to die in shorter times than VSD-H ($P<0.0001$). Results demonstrated steam can be an effective "plus" for VSD+ depopulation of laying hens in a cage-free system. While experiments emulated a commercial poultry environment, applications of techniques in present study still need to be evaluated in a commercial egg-laying facility.

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## LIST OF ABBREVIATIONS

**AAAP:** American Association of Avian Pathologists

**AVMA**: American Veterinarian Medical Association

**AWI**: Animal Welfare Institute

**$CO_2$**: Carbon dioxide

**FAD**: Foreign Animal Disease

**HPAI**: Highly Pathogenic Avian Influenza

**$PaCO_2$**: Arterial Blood Partial Pressure of Carbon Dioxide

**VSD**: Ventilation Shutdown only

**VSD+**: Ventilation Shutdown plus heat and/or $CO_2$

**USDA**: United States Department of Agriculture

**USDA-APHIS**: United States Department of Agriculture- Animal, Plant Health Inspection Service

**USDA-ERS**: United States Department of Agriculture- Economic Research Service

**UV light:** Ultraviolet light

# CHAPTER 1

# REVIEW OF THE LITERATURE

## LITERATURE REVIEW

### *Cage-Free Market*

The U.S public, restaurants, and food retailers have mandated changes to laying hen housing systems, moving away from conventional cages to alternative systems, by means of legislation and commercial channels (Ochs et al., 2019). In these alternative systems, hens have the ability to express natural behaviors and are provided with additional resources that are thought to improve hen welfare (Mellor and Webster, 2014; Campbell et al., 2016; Hartcher and Jones, 2017). In the U.S.A, ten states have passed legislation requiring cage-free systems to replace conventional cages, included are Arizona, California, Colorado, Massachusetts, Michigan, Nevada, Oregon, Rhode Island, Utah, and Washington (Johnson, 2022). Furthermore, pledges by large egg buyers such as McDonalds, Starbucks, Walmart, and well beyond 200 restaurants, supermarkets and other companies have committed to discontinue purchasing eggs produced in conventional cages and use only cage-free eggs (Graber and Keller, 2020; Trejo-Pech and White, 2021).

According to United Egg Producers, in March 2021, organic and cage-free shell egg production accounted for 29.3% of current table egg layer flock. The United States Department of Agriculture (USDA) released Cage-Free Shell Egg Report on December 1, 2022 and estimated approximately 34.6% of the total U.S table egg layer flock was in cage-free production. However, to meet projected demand, approximately 60% of U.S. table egg flock must be in cage free production by 2025 (O'Keefe, 2023).

### *Labor in Cage-Free Systems*

Anderson (2014) concluded that moving from intensive (cage) to extensive production systems requires substantial increases in time commitments, particularly, when shifting from cages to cage-free production there was a 45% increase in man-hours (labor). A discussion held with representatives from four major egg producers concluded that three to five times more labor is required to manage cage-free hens when compared with conventional housing systems (O'Keefe, 2018). Labor challenges arise for cage-free systems, particularly aviary systems, during depopulation especially if birds must be caught prior to depopulation. Catching for removal may be needed if water-based foam method or gas are utilized since cost and logistics are proportionally related to the amount of area that must be treated (AVMA, 2019). If birds

need to be caught to reduce treated area (i.e., reduce cost), aviary systems encourage hens to freely move through the system, hens may be scattered throughout and will need to be chased and caught individually (Knowles and Wilkins 1998; Gerpe et al., 2021). Access to hens inside an aviary can be problematic for workers, but also stressful on hens since unintended injury can occur due to attempt to escape and may crash into housing equipment (Knowles and Wilkins 1998; Gerpe et al., 2021).

### *Avian Vision*

In avian species, the eye is arguably the most important sensory organ, eyes occupy much of cranial space, optic nerve is one of the largest cranial nerves, and avian eyes have remarkable ability (Jones et al., 2007; Korbel, 2012; Seifert et al., 2020). The avian eye includes various unique anatomical features, most notably an ultraviolet (UV)- translucent lens and UV-specific retinal cones (Korbel, 2012). Chickens, including laying hens, possess four cone photoreceptors to detect blue light, green light, red light, and ultraviolet light that provide tetrachromatic vision (Burkhardt, 1982; Wilby et al., 2015; Seifert et al., 2020; House et al., 2020b). Each retinal cone also contains oil droplets, which filter incident light before it reaches visual pigments within cones and ultimately reduces spectral overlap between pigments, increasing the amount of color a bird can perceive (House et al., 2020b). When studying individual cone sensitivity in chickens, Wilby et al., (2015) found violet and blue cones had higher sensitivity compared to red and green cones due to an influence of ellipsoid and oil droplet.

### *Light Flash and Effects on Poultry*

Having a correct lighting program throughout the production cycle of domestic poultry species is crucial for producers. For this reason, lighting is of particular interest to researchers as there is an economic benefit to producers since feed consumption and growth rate can be influenced by light. Researchers have attempted to improve feed efficiency and growth rate through addition of light flashes throughout production cycle of broilers and Egyptian dual-purpose breeds (Farghly, 2014; Fargly and Makled 2015). Fargly et al., (2017), investigated effects of applying light flashes during rearing of 360 naked neck (Sharkasi) birds (growing and laying periods). Concluding points from the study were that birds subjected to 10, 20, or 30-min of light flashes per hour had an overall better growth performance, egg production, shell

thickness, and livability as opposed to birds reared under common light (12-h during growing and 16-h during laying), 40-min and 50-min of light flashes per minute (Farghly et al., 2017).

### *UV Light Spectrum and Effects on Poultry*

Ultraviolet light is composed of shorter wavelengths (100-400 nm) of electromagnetic radiation spectrum and is divided into three parts: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm) (Rana and Campbell, 2021). Lights emitting UVA and UVB may be used as a management tool to improve welfare and health of poultry species, whereas UVC wavelengths can protect poultry species against airborne viral infections, but continuous exposure can cause corneal damage (Lewis and Gous, 2009). In a study by Sobotik et al., (2020), researchers found that rearing laying hens with UV lights (380 nm) can reduce stress and fear responses but found no difference between lighting treatments in production parameters. However, Spindler et al., (2020) found no benefit supplementing 4-5% UVA light to laying hens due to a higher incidence of plumage damage and skin injuries, although authors expressed this could had been attributed to higher light intensity, genetic aspects, nutritional imbalances, housing aspects and management factors. Pekin ducks and broilers reared under an environment illuminated with both LED bulbs and UV light supplementation had decreased stress and fear responses and improved feather condition (James et al., 2018; House et al., 2020a; House et al., 2020b). During a preference test, laying hens between 6 and 21 weeks of age preferred daylight (combination of UV and white light) and forest light (combination of UV, blue, green, and red light) compared to conventional light used in poultry housing and had higher frequencies for locomotion, standing and foraging behaviors (Wichman et al., 2021). When domestic poultry species were exposed specifically to UVB, there was an improvement in skeletal health, egg production, synthesis of vitamin D3, and higher content of vitamin D in egg yolk (Lietzow et al., 2012; Schutkowski et al., 2013; Kühn et al., 2019; Wei et al., 2020; Rana and Campbell, 2021).

### *Notifiable Foreign Animal Disease*

Threat of a foreign animal disease (FAD), specifically an outbreak of a highly contagious disease, would have severe repercussions to the United States and the world. Some consequences identified by U.S Department of Agriculture (USDA) included, “social and psychological impact on both producers and consumers, disruption of food supply, threatening of animal health and

animal agriculture, and economic losses attributed to loss of international trade and eradication efforts" (USDA-APHIS, 2015). For this reason, U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) compiled a list of notifiable diseases for different animal species, based on a list released by the World Organization for Animal Health, that must be immediately reported by an animal health professional (i.e., veterinarian, diagnostic laboratorian, trained technician, etc.) (USDA-APHIS, 2022a). For avian species, the following diseases constitute as a notifiable disease: duck viral hepatitis, fowl typhoid, highly pathogenic avian influenza (HPAI), low pathogenic avian influenza (LPAI), pullorum disease, turkey rhinotracheitis, and virulent Newcastle disease (USDA-APHIS, 2022a). When responding to a FAD outbreak in the United states, there are three critical goals that must be achieved so normal production can resume: "1) detect, control, and contain the disease in animals as quickly as possible; 2) eradicate the disease using strategies that seek to protect public health and the environment, and stabilize animal agriculture, the food supply, and the economy; and 3) provide science- and risk-based approaches and systems to facilitate continuity of business for non-infected animals and non-contaminated animal products" (USDA-APHIS, 2021).

***What is Mass Depopulation and How is it Used?***

Mass depopulation "refers to the rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable" (AVMA, 2019). Mass depopulation differs from what the American Veterinary Medical Association (AVMA) has defined for euthanasia, which describes "ending the life of an individual animal in a way that minimizes or eliminates pain and distress; a good death is tantamount to the humane termination of an animal's life" (AVMA, 2020). Knowing the distinction between these two terms is critical because said difference determines what procedures will be utilized under specific circumstances. Mass depopulation is reserved to prevent or relieve animal suffering (based on AVMA criteria) under urgent circumstances and may include natural disasters when poultry housing may be damaged or essential services are not available in time; non-natural disasters scenarios (i.e., terrorism, bioterrorism, accident, etc.); contamination of food and/or water sources; pandemic diseases; poultry reportable diseases; highly infectious avian diseases;  and severe market or infrastructure disruptions (AAAP, 2021). In recent years, several events have caused mass depopulation of commercial poultry species. In

December 2014, the United States identified a positive case of highly pathogenic avian influenza (HPAI) that would later turn into an HPAI outbreak, ending in June 2015 (USDA-APHIS, 2016). This specific outbreak resulted in "depopulation of 7.5 million turkeys and 42.1 million egg-layer and pullet chickens, with devastating effects on these businesses, and a cost to Federal taxpayers of over $950 million" (USDA-APHIS, 2016). Many lessons were learned during 2014 to 2015 outbreak that would facilitate the United States preparation for future HPAI cases, particularly improving ability of animal health officials to begin depopulation within 24 hours of a positive detection to decrease spread of virus (USDA-APHIS, 2016).

While models exist to predict potential impact of pandemics on humans, effects of COVID-19 pandemic on livestock and poultry production, and food supply chain were unexpected (Marchant-Forde and Boyle, 2020). During the pandemic, certain processing plants ceased operation due to disease outbreaks amongst workers. Processors then had to take adequate time to disinfect premises for workers to safely return (Marchant-Forde and Boyle, 2020; McCarthy and Danley, 2020). At this time more people were purchasing food supplies from supermarkets to cook at home, but there was decreased demand for poultry products from restaurants and foodservice businesses resulting in the need for less poultry to be processed (Sharma et al., 2020). A combination of decreased demand, and processing plant closures resulted in depopulation of 2 million broilers, and 61,000 laying hens (Kevany 2020).

Beginning in 2022 the United States experienced another extensive HPAI outbreak. Virus was first confirmed in Canada by December 2021, by January 2022 HPAI was first confirmed in wild birds, in the United States; and by February, first detection in commercial flock (Graber, 2022). Despite U.S. and Canada producers establishing exceptional biosecurity measures during this time, the outbreak has lasted a minimum of one year and "a minimum of 300 commercial flocks in the U.S. have been affected by the virus, involving about 55.9 million birds" at time of writing (Graber, 2022).

### *Current Acceptable Mass Depopulation Methods*

In 2019, AVMA released a guideline for depopulation of livestock and poultry. Approved methods fall under two categories, "preferred methods" and "acceptable methods under constrained circumstances". Poultry methods are approved based on housing methods of individual farms (AVMA, 2019; Marchant-Forde and Boyle, 2020). Preferred depopulation

methods for poultry are water-based foam generators or nozzles; whole house, partial house, or containerized gassing; cervical dislocation, mechanical assisted cervical dislocation; and captive bolt gun (AVMA, 2019). Additional acceptable methods under constrained circumstances include: VSD+, controlled demolition, exsanguination, and decapitation (AVMA,2019). Based on AVMA depopulation guidelines and recommendations from American Association of Avian Pathologists (AAAP) for mass depopulation of poultry flocks, methods should be assessed based on three criteria: 1)" ability to induce loss of consciousness followed by death in a timely manner with a minimum of pain or distress, 2) reliability and irreversibility of the method to result in death of the animal, and 3) compatibility with the safety of humans, other animals, and the environment" (AAAP, 2021). For example, flocks housed in metal structures (i.e., cages and aviaries) are unable to utilize the water-based foam as a preferred method due to loss of integrity of the water-based foam; discussion of other methods will occur in a separate section of thesis. For this reason, AVMA does not recommend usage of water-based foam generators and nozzles in commercial facilities with metal structures nor does not recommend use of guns or VSD alone to depopulate flocks (AVMA, 2019).

***Ventilation Shutdown Plus (VSD+)***

To avoid extended time of death of birds affected with HPAI and minimize spread of virus, USDA-APHIS developed a 24 to 48-h "stamp out" policy for depopulating flocks (USDA-APHIS, 2015). Under constrained circumstances, where ability to obtain resources necessary for preferred depopulation methods (i.e., clean water sources, specialized equipment, carbon dioxide, and trained workers) within allowable time frame, ventilation shutdown plus heat and/or $CO_2$ (VSD+) has been approved as a last resort method under state officials and USDA veterinary supervision (Gingerich, 2015).

Ventilation shutdown (VSD) was coined by the United Kingdom Department for Environment, Food, and Rural Affairs (DEFRA) in 2006 "when making contingent plans to combat sudden and serious outbreaks of disease dangerous to poultry handlers and to the general public" (RSPCA, 2008). The UK was first to pass a legal amendment to their Welfare of Animals (Slaughter and Killing) regulations of 1995, in 2006 recognizing VSD usage to depopulate flocks under exceptional circumstances (BBC, 2006). While the United States, utilization of VSD as a depopulation method was not considered until after the 2014 to 2015

HPAI outbreak (USDA-APHIS, 2015). Over the years, accidental ventilation failure on farms has caused unintentional mass mortality so, hyperthermia death loss caused by ventilation shutdown is not a new concept (Rivenbark, 2010; Chow and Chan, 2015; ABCNews, 2015; Hallare, 2020).

VSD is defined as "closing up the house, shutting inlets, and turning off the fans; body heat from the flock raises temperature in the house until all birds die from hyperthermia" (AVMA, 2019). However, VSD alone is not recommended since it does not lead to unconsciousness quickly and addition of some "plus" is required, thus AVMA guidelines recommend addition of heat and/or carbon dioxide to ensure 100% mortality is achieved, this is referred to as VSD+. The USDA released a HPAI response guide for using VSD+ to control HPAI and states that indoor temperature of house/barn should be raised as quickly as possible to 40°C or higher and maintained between 40°C and 43.3°C for a minimum of three hours (USDA-APHIS, 2022b). DEFRA has a similar time and temperature requirement for VSD+, but goes a step further and states that, if possible, the relative humidity of the bird house/barn be maintained at 75% or higher for the three-hour minimum (DEFRA, n.d.).

***Current Research on VSD+***

Evaluation of ventilation shutdown in a multi-level caged system, conducted by Eberle-Krish et al., (2018), was the first of its kind to explore VSD plus $CO_2$ to depopulate laying hens housed in a conventional- caged structure. Researchers were able to achieve 100% mortality in 90-min by addition of carbon dioxide and a relative humidity of 80% to 88.9%; and in 120-min with supplemental heat and a relative humidity of 73% to 84%. However, VSD alone was unable to reach 100% mortality after 225-min of implementation. Based on this research, AVMA included carbon dioxide as an appropriate "plus" for VSD+ depopulation in 2019 depopulation guidelines. Zhao et al., (2019) used computer models to determine the best methods for VSD+ in various poultry housing systems. In this experiment, researchers were able to model indoor environment and supplemental heat requirement for VSD in three types of hen housing (i.e., manure belt cage, high-rise cage, and cage-free); the model was later validated by conducting a VSD depopulation in a layer breeder house (i.e., floor reared/cage-free house). When comparing predicted indoor temperature and $CO_2$ concentrations obtained from the model to the measured values, the temperature and $CO_2$ concentrations agreed while predicted relative humidity was

less than 5% off measured relative humidity (Zhao et al., 2019). Additionally, models indicated supplemental heat requirement will differ among housing types due to differences in stocking density, air leakage of house or how tight house is sealed, and ambient temperature (Zhao et al., 2019). Researchers confirmed leading cause of death was hyperthermia and not hypoxia based on post-mortem examination after VSD validation test and suggested having a high relative humidity will expedite hyperthermic process (Zhao et al., 2019). Birds' behavior in the VSD validation test were observed and after 135-min into VSD birds started lying down, with 95% lying down after 180-min (Zhao et al., 2019).

The Animal Welfare Institute (AWI) compiled a document with information obtained through the Freedom of Information Act regarding 2022 USA HPAI outbreak, for flock depopulations from February to October 2022. Within this time frame, a total of 256 commercial flocks were depopulated, totaling approximately 46,670,600 birds (AWI 2022). Analyzing number of flock depopulations by method revealed: utilization of $CO_2$ cart or container only depopulated 10 flocks (3.9%); $CO_2$ whole-house gassing depopulated 2 flocks (0.8%); foam depopulated 105 flocks (41.0%); foam and another method (other than VSD+ heat) depopulated 12 flocks (4.7%); other methods depopulated 11 flocks (4.3%); VSD+ heat depopulated 44 flocks (17.2%); VSD+ heat and $CO_2$ depopulated 15 flocks (5.9%); VSD+, $CO_2$ and other method depopulated 3 flocks (1.2%); VSD+ heat and foam depopulated 45 flocks (17.6%); VSD+, foam and other method depopulated 2 flocks (0.8%); and VSD+ heat and other method depopulated 7 flocks (2.7%) (AWI 2022). When other methods were utilized, it included captive bolt gun, manual cervical dislocation, and mechanical cervical dislocation (KEDS) (AWI, 2022). However, breaking down the number of birds depopulated by method uncovered that most birds were depopulated with VSD+ heat and $CO_2$ (23,465,500), foam depopulated a total of 4,410,900 birds, VSD+ heat depopulated 9,420,400 birds and $CO_2$ whole-house gassing depopulated 112,200 (AWI 2022).

***Challenges of Mass Depopulation in Cage-Free Systems***

Challenges arise for cage-free systems, particularly aviary systems, if birds must be caught prior to deployment of depopulation methods. This may be the case if water-based foam method or gas are utilized since cost and logistics are proportionally related to the amount of area that must be treated (AVMA, 2019). If birds need to be caught in order to reduce the treated area

(i.e., reduce cost), aviary systems encourage hens to freely move through the system, hens may be scattered throughout and will need to be chased and caught individually (Knowles and Wilkins 1998; Gerpe et al., 2021). Access to hens inside an aviary can be problematic for workers, but also stressful on hens since unintended injury can occur due to attempt to escape and may crash into housing equipment (Knowles and Wilkins 1998; Gerpe et al., 2021).

### *Carbon Dioxide Shortages, and Limitations in Cage-Free Systems*

Due to associated cost and current supply shortage, $CO_2$ usage for mass depopulation purposes may become a challenge. Currently, the United States of America is experiencing a national $CO_2$ shortage due to several factors. Reasons included are contamination at largest natural $CO_2$ production hub, planned and unplanned ammonia plant closures for maintenance, decreased fuel demand and production during COVID-19 pandemic, and labor shortages in bulk transportation (Greenwood 2020; Clouse 2022; Taylor 2022; Chappell 2022).

Carbon dioxide is denser than air, so if used in a cage-free system, a higher concentration would be found in floor area compared to the rest of barn. This can be a problem because chickens can detect $CO_2$ concentrations at very low levels (5.0%-7.5%) and will actively choose to avoid inhaling air with 60% $CO_2$, meaning birds might move from floor area to higher tiers of aviary (Raj and Gregor 1991; Gerritzen et al., 2007; Sandilands et al., 2011). Removal of carcasses within aviary systems might be difficult due to rigor mortis or stiffening of muscles, of most concern here are leg and foot muscles, and birds may "grasp" metal structure (Duncan 2001; producer interactions). Environmental temperatures may also affect the onset of rigor mortis. Research has shown that animals dying in a hotter environment, such as temperatures observed in VSD+, would encounter rapid chemical changes as opposed to a colder environment thus decreasing time to onset of rigor mortis (Mesri et al., 2017).

### *Heat Stress and Thermoregulation in Poultry*

Commercial poultry species, like other avian species, are considered homeothermic animals (Donald and Williams, 2001; Anderson and Carter, 2004; Oloyo, 2018). Homeothermic animals can maintain a constant core body temperature. For chickens core body temperature can be maintained between 40°C and 42°C, when environmental temperatures are within the thermoneutral zone (18°C to 24°C); although species, sex, breed, feather coverage, and age will

dictate the range of the thermoneutral zone (Fox, 1951; Hutchinson and Sykes, 1953; Donald and William, 2001; Anderson and Carter, 2004). Poultry, however, are not well adapted to high ambient temperatures as they lack sweat glands to remove excess heat through sweating and the presence of feathers inhibits heat loss since feathers function as a form of insulation (Bhadauria et al., 2014; University of Kentucky, n.d.).

When environmental temperatures are within the thermoneutral zone or below, birds will use sensible heat loss (i.e., radiation, conduction, and convection) to dissipate excess body heat to surrounding air (Anderson and Carter, 2004; Daghir, 2008; Bhadauria et al., 2014) However, the proportion of heat loss through radiation, conduction, and convection depends on the temperature difference between the bird and their environment (Anderson and Carter, 2004). While head appendages (e.g., comb) aid in direct heat loss, at warmer temperatures it becomes critical for birds to dissipate body heat to surrounding environment (Anderson and Carter, 2004; Bhadauria et al., 2014). When temperatures continue to increase outside the thermoneutral zone, the method of heat loss shifts from sensible heat loss to latent heat loss (i.e., respiratory evaporative cooling/panting) (Anderson and Carter, 2004). Refer to **Table 1.1** for detailed description of heat loss methods and direction of heat flow.

| **Sensible Heat Loss** | | **Direction of Heat Flow** |
|---|---|---|
| | **Radiation**- Flow of thermal energy without aid of a material medium between two surfaces | All surfaces radiate heat and receive radiation back; net radiation heat flow is from higher to lower temperature surfaces |
| | **Conduction**- Thermal energy flow through a medium or between objects in physical contact | Direction of energy transfer depends on a temperature gradient; heat moves from areas of higher to lower temperature |
| | **Convection**- Heat flow through a fluid medium such as air; thermal energy moves by conduction between a solid surface and layer of air next to the surface, and thermal energy is carried away by the flow of air over the surface | Energy transfer to the air depends on temperature and movement of air across skin surface; heat is transferred to air moving across the skin surface if air is at a lower temperature than the skin |
| **Latent Heat Loss** | | **Direction of Heat Flow** |
| | Evaporation- Transfer of heat when a liquid is converted to a gas; when water is converted from a liquid to a vapor, heat is utilized | Energy transfer is influenced by the relative humidity, temperature, and air movement; heat is transferred from animal's body to water, turning it to water vapor |

**Table 1.1.** Methods of Sensible and Latent Body Heat Loss
**Source: Anderson, K.E. and Carter, A.T. (2004). Hot weather management of poultry. The Poultry Site.**

Respiratory evaporative cooling occurs by vaporization of moisture from the damp lining of the respiratory tract (lungs and air sacs) (Donald and William, 2001; Saeed et al., 2019). Under heat challenges, birds will increase their respiration rate through open mouth breathing and undergo through two types of thermal panting (referred to as thermal tachypnea or thermal polypnea) (Whittow, 2000). In type I panting, "the increased ventilation responsible of achieving increased respiratory water loss is achieved by augmentation of increased respiratory frequency sufficiently to overcome the effect of a concurrent reduction in respiratory tidal volume" (Whittow, 2000). However, as the core body temperature of the bird continues to increase to high levels, respiratory frequency decreases, and switches to type II panting; type II panting "eventually declines as the limit of the animal's thermal tolerance is approached" (Whittow, 2000). Using thermal panting to reduce core body temperature can be a vigorous activity, especially at higher humidity levels, for this reason many birds supplement thermal panting with rapid fluttering of the gular area (i.e., gular flutter) (Whittow, 2000).

Randall and Hiestand, (1939), were the first to observe the panting mechanism and heat stress symptoms in chickens. Some behavioral observations seen alongside panting were beak sporadically opening and closing, raised head, and often accompanied by defecation with abundant loss of water (Randall and Hiestand, 1939). Additionally, researchers noted that if a high body temperature was maintained for a prolonged time, panting rate would reach a maximum point and lose effectiveness (Randall and Hiestand, 1939). Other studies showed that at ambient temperature of 32°C and relative humidity of 50% to 60%, hens reached maximum ability to dissipate heat through evaporative cooling (Barrot and Pringle, 1941; Wilson, 1948). While panting is effective at removing excess body heat for short durations of time, there are physiological consequences if birds pant for extended lengths of time.

Prolonged increase in respiration rate (i.e., prolonged panting) may lead to respiratory alkalosis (Mather et al., 1980; Ahmad and Sarwar, 2006). Respiratory alkalosis is a condition that occurs when blood pH is increased above normal range and becomes more basic, as a result from increased respiration rate. Panting causes an excessive loss of $CO_2$ from the body which reduces the partial pressure of $CO_2$ in blood (Ahmad and Sarwar, 2006; Goel 2020). This causes a change in the bicarbonate to $CO_2$ ratio, with more bicarbonate being present; $CO_2$ is considered the acid, while bicarbonate is considered the base (Franco-Jimenez et al., 2007). In turn, bicarbonate buffer system decreases concentration of hydrogen ions, and since there is more

bicarbonate present in the blood, this results in an increase in blood pH (Mongin, 1968; Richards, 1970; Ahmad and Sarwar, 2006). Additionally, this rise in pH leads the kidneys to excrete excessive amounts of electrolytes (i.e., sodium, potassium, and chloride) to aid in respiratory alkalosis (Anderson and Carter, 2004). However, respiratory alkalosis may be avoided when birds use gular flutter or a slower, deeper breathing pattern (i.e., compound ventilation) (Ramirez and Berstein, 1976; Whittow 2000).

Mather et al., (1980) compared cockerels panting frequencies, arterial blood pH (pHa), and arterial blood partial pressure of carbon dioxide ($PaCO_2$) in three groups exposed to 45°C to develop acute hyperthermia (control—cockerels developed severe alkalosis; $CO_2$—$CO_2$ introduced to maintain $PaCO_2$ at pre-hyperthermic values; and acid infusion—addition of HCl to maintain pH at pre-hyperthermic values). For respiratory frequencies, all groups had a rapid increase, approximately 140 breaths/min as body temperature increased. Additionally, as control group reached body temperature of 42.0°C, respiratory rated increased to approximately 240 breaths/min since birds developed severe respiratory alkalosis; $CO_2$ group did not breathe faster than 152 breaths/min; and acid infused group reached 200 breaths/min when body temperature was 43.5°C (Mather et al., 1980). Control cockerels with severe alkalosis had decreased $PaCO_2$ (27.2 torr to 15.2 torr) and increased pHa (7.48 to 7.64) when body temperature reached 45°C; $CO_2$ group needed about 28 torr $PCO_2$ to maintain constant $PaCO_2$ (28 torr) and pHa (7.47); and acid infusion group had a pHa between 7.39 and 7.45 when body temperature increased to 41.5°C and $PaCO_2$ decreased, but not as low as control group (Mather et al., 1980). Dissipation of heat by evaporative cooling demands an increase in respiration, while respiratory alkalosis demands a decrease in respiration (Daghir, 2008). If birds are unable to lower core body temperature, and environmental temperatures reach an upper critical temperature of 35°C, heat stress occurs (Saeed et al., 2019; University of Kentucky, n.d.).

Lara and Rostagno, (2013), defined heat stress as a result from "a negative balance between the net amount of energy flowing from the animal's body to its surrounding environment and the amount of heat energy produced by the animal". Heat stress can be chronic or acute (Saeed et al., 2019). Acute heat stress results from abrupt and quick periods of extremely high temperature and humidity; while chronic heat stress is an outcome of an extended period of elevated temperature and high humidity (Kettlewell et al., 1993; Scanes, 2015). To determine how successful birds will be combating heat stress, four climatic elements should be

considered—air temperature, humidity, air movement, and radiant energy (Lee et al., 1945). High temperature coupled with high humidity can have severe repercussion to birds' health, if not managed properly (Donald and Williams, 2001; Holik, 2009). At an ambient temperature of approximately 29.4°C and a relative humidity of approximately 50%, birds can effectively balance body heat through evaporative cooling (Donald and Williams, 2001). As relative humidity increases above 70%, the efficiency of evaporative cooling decreases due to reduction in the amount of moisture that can be evaporated from birds' respiratory tract (Donald and Williams, 2001).

When poultry are exposed to high ambient temperatures and/or high relative humidity, birds will adjust their behavior and physiological needs to combat heat stress (Daghir, 2008; Bhadauria et al., 2016). Adjustment of behavior can occur more rapidly than physiological adjustments and at less of a cost to the bird (Daghir, 2008). In a study conducted by Mack et al., (2013), two strains of White Leghorn hens (Dekalb XL, and kind gentle bird) were used to evaluate productivity and behavior following heat stress; mean hot temperature for heat stress group was set to 32.6°C, control was 24.3°C and humidity was similar between groups. Hens subjected to heat stress spent more time drinking, panting, resting, as well as more time with their wings elevated; however, heat stressed hens spent less time feeding, moving, and walking (Mack et al., 2013). Lifting wings away from birds' body exposes an unfeathered (i.e., apteria) area under wings, which aids in reducing body temperature (Gerken et al., 2006). If birds are unable to combat hyperthermic effects and body temperature reaches the upper lethal temperature of 47°C, death is inevitable (Donald and Williams, 2001).

### *Evaluation of Insensibility and Death*

When determining whether an animal is insensible, postmortem investigations and signs are most often used for assessment. Examples of physical measures for assessing insensibility in birds include absence of neck tension, sustained eye closure, no blinking/nictitating membrane reflex, and absence of breathing (Eramus et al., 2010). Once insensibility signs have been confirmed, it is crucial to ensure insensibility is irreversible and now death can be confirmed. Signs utilized to determine time of death include absence of pulse or heartbeat; loss of posture; occurrence of feather erection coupled with neuromuscular spasms that are followed by absolute relaxation of muscles (Raj et al., 1998; European Food Safety 2004; Gerritzen et al., 2007).

Neuromuscular spasms may be divided into two phases, “clonic phase characterized by vigorous wing flapping, and tonic phase characterized by stillness with legs and wings outstretched” (Raj et al., 1990; Eramus et al., 2010). After cessation of neuromuscular spasms, there will be final paddling motions leading to relaxation and death (Raj et al., 1990; Eramus et al., 2010).

### *Forced Air Heaters and Steam*

Forced air heaters use a heat source powered by propane, natural gas, or electricity to raise the temperature of a volume of air (Biermeier et al., 2022). Then, heated air is pushed out to the environment by means of a powerful fan or blower. When air temperature increases, relative humidity of the environment decreases; conversely when temperature decreases, relative humidity increases (Bencloski, 1982). In a short simulation, Bencloski, (1982), demonstrated the inverse relationship between temperature and relative humidity; at a temperature of 4.4°C relative humidity was 100%, while when temperature was increased to 32.2°C, relative humidity decreased to 19%. Additionally, this relationship was observed in an experiment conducted by Eberle-Krish et al., (2018). When only a heater was utilized in VSD, the relative humidity of room decreased over time while temperature increased. Although relative humidity decreased in room when researchers used $CO_2$ in VSD, it did not decrease as observed in VSD with heat; while when only VSD was utilized, relative humidity of environment increased in a comparable manner to temperature. Conventional cages used in this study were completely enclosed from both sides, creating a chamber; the chamber was then sealed with a 10-mil polyethylene plastic ceiling. This type of environment allowed for relative humidity to be high due to tightly sealed chamber and ventilation of room being off, thus moisture given off by birds (i.e., gular flutter) could not escape. Creating and tightly sealing such chamber may be a challenging task in a commercial barn due to the large size.

Utilization of forced air heaters is helpful raising ambient temperature, but relative humidity is affected since warm dry air is pushed out, by means of the fan located within the heater, thus reducing the overall moisture of the environment, and lowering relative humidity. Steam can provide both high temperature and moisture and has been a longtime resource used for space heating in commercial buildings and homes (Robbins, 2021). Additionally, steam heating can generate comparable heat loads to direct fired heating, yet it can do it in a more controlled form, creating a better heating consistency (Weil-Mclain, 2020). Businesses can

coordinate their unique equipment needs based on what capacity of steam heating is needed, for this reason high- and low-pressure steam boilers are available. Weil-Mclain, (2020), indicated that with low-pressure steam heating, “the steam from the boiler is pushed out to the building at a low pressure as opposed to high”. Some advantages to using low-pressure steam heating instead of high-pressure steam heating include same load of steam can travel faster, cheaper equipment, reduced regulatory burden, increased staffing flexibility, and reduced operating and insurance costs (Weil-Mclain, 2020).

Moreover, inclusion of steam and high temperature during VSD+ has been explored in swine depopulation (Baysinger et al., 2021). During the Covid-19 pandemic, processing plant closures led to overcrowding of pigs in farms located in the Midwest region of the United States. Farm management discussed several mitigation strategies “to avoid animal welfare issues associated with overcrowding and decrease the risk that depopulation would be required” (Baysinger et al., 2021). As veterinarians and farm management evaluated each preferred methods and approved methods under constrained circumstances for swine depopulation, VSD+ with heat seemed the most feasible for this company (Baysinger et al., 2021). However, during a proof-of-concept trial, VSD with only heat did not meet AVMA depopulation guidelines recommendations (>95% mortality in <1 h) (Baysinger et al., 2021). For this reason, veterinarians, academics, and engineers determined that increasing humidity in barn would expedite hyperthermic process in VSD+, and addition of steam would achieve this (Baysinger et al., 2021). Results obtained from case study exceeded AVMA depopulation guidelines of more than 95% mortality rate in less than one hour (Baysinger et al., 2021).

***Conclusion***

Using bird physiology and behavior to our advantage should be of interest when utilizing UV lights to elicit movement responses in birds and improving VSD+ for mass depopulation.

Managing birds in cage-free systems can be challenging since it requires three to five times more labor than in a conventional setting. For this reason, the table egg industry is looking elsewhere (i.e., robots) to help alleviate the workload of workers. However, such automation may not be suitable or usable during a mass depopulation event. Being able to gather hens, reared in multi-tier cage-free systems, in floor area and out of the system may be easier on workers in terms of conducting welfare checks; this may also be the case when performing a mass depopulation procedure. Depending on what method is utilized for mass depopulation, workers may have to individually catch hens to reduce treated area, and this may be challenging in systems where hens can freely move. Yet, if workers do not have to catch hens prior to depopulation, carcass removal from cage-free systems (i.e., aviaries) becomes difficult since bodies stiffen by rigor mortis may be seen grasping onto the structure itself. For this reason, finding a solution that may alleviate the workload during depopulation should be of interest. Since UV lighting has been used to enhance health and welfare of birds, it could potentially be used as a non-stress inducing tool to elicit a movement response on birds, particularly as a flash of light. By concentrating hens in floor area and out of the system, depopulation may be less challenging in cage-free systems.

Mass depopulation is reserved for emergency situations (disease outbreaks, market disruptions, natural disasters, etc.) when the death of the animals is unplanned. While methods exist to ensure depopulation is conducted in a manner in accordance with AVMA and USDA guidelines, some of the approved methods may be challenging to utilize due to availability of resources and safety concern of workers involved in depopulation. Since VSD+ is not a preferred depopulation method (i.e., not the first method utilized), refinement of method is needed given that it is not widely used due to being last choice on depopulation methods list. For this reason, more research must be conducted in VSD+ to improve the method up to a standard that is just as efficient as the gold standard (VSD+ $CO_2$ with heat). Steam and heat addition to a VSD+ procedure have been explored in swine depopulation during a case study. Results obtained from case study demonstrated how effective VSD+ with steam and heat were at depopulating swine as more than 95% mortality rate was attained in less than one hour (exceeding AVMA

recommendations). Since steam and heat proved to be suitable “pluses” for VSD+ in swine, this combination may be effective in poultry depopulation, particularly in cage-free systems. Steam will have the potential to provide both high heat and humidity which will hinder how effectively birds will dissipate heat through evaporative cooling (i.e., panting). If birds are unable to remove excess body heat in a timely manner, death will be the result. Addition of steam and heat to VSD+ could have the potential to expedite the depopulation process and be aligned with AVMA and USDA expectations (i.e., “stamp out” in 24 to 48 h).

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# CHAPTER 2

## CAN UV LIGHT INDUCE MOVEMENT IN CAGE-FREE LAYING HENS?

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## SUMMARY

In recent years, more farms have been transitioning to cage-free systems as multiple states passed regulations banning use of conventional cages, and several large food service companies pledged to exclusively source cage-free eggs by 2025-2026. However, transition and management of hens within new cage-free systems has been problematic. Additionally, catching hens prior to mass depopulation or carcass removal after depopulation becomes a challenging task for workers in these systems. The goal of study was to explore 10-sec flashes (2 pulse/d, 1 pulse in AM and 1 pulse in PM) of UV light and darkness as management tools to stimulate a movement response from laying hens, with the aim of moving hens from within the aviary system to floor area. Approximately 1,800 Lohmann Brown hens were divided into 4 rooms equipped with Big Dutchman Natura 60 aviary; 150 hens were placed into 3 sections of aviary, totaling 450 hens per room. Six UV-light bars in 395 to 400 nm wavelength were used. Four lighting treatments were as followed: 1) Control, 2) UV light flashed for 10-sec (UV), 3) floor area was darkened (DF), and 4) UV flashed for 10-sec plus floor area darkened (DF+UV). Each treatment was applied once in AM and once in the PM. Videos were recorded to assess hens' spatial distribution (difference in number of hen pre- and post-treatment application) and behavior (preening, dust bathing, wing flapping, perching, and standing alert), before and after treatments were applied. Results demonstrated that when UV flash was combined with darkened floors in AM application, a greater difference in number of hens was observed in this treatment compared to other lighting treatments; while in PM application this difference was only observed when comparing DF+UV to control. UV light flashes influenced hens' behavior, with more stress related behaviors (standing alert) apparent in treatments where UV lights were used, while more hens exhibited normal behaviors (preening) in non-UV light treatments. Based on these observations, a flash of UV light was successful in moving laying hens out of aviary and onto floor area, but this was only effective for 6-min and may not be effective for long-term movement.


## DESCRIPTION OF PROBLEM

Through legal and market channels, U.S. consumers, food retailers, and restaurants have mandated changes to laying hen housing systems, moving away from conventional cages to alternative systems (Ochs et al., 2019). The additional resources provided in alternative non cage systems are known to improve hen welfare by promoting hens' ability to express natural

behaviors (Mellor and Webster, 2014; Campbell et al., 2016; Hartcher and Jones, 2017). The World Organization for Animal Health's (OIE) definition of animal welfare refers to how well an animal can cope within its living conditions and is comprised of both physical and mental health (OIE, n.d.). Furthermore, pledges by small and large egg buyers such as fast-food chains and large grocery store chains, have committed to discontinue purchasing eggs from cage raised hens and replace them with cage-free eggs; however, certain company pledges may fail to meet deadlines (Trejo-Pech and White, 2021). According to United Egg Producers, as of March 2021, organic and cage free shell egg production accounted for 29.3% of the table egg layer flock, while most of the U.S. layer population was in conventional cages (70.7%). To meet projected demand, approximately 66% of U.S. hens must be in cage free production by 2026 (United Egg Producers, n.d.).

Over the last few decades, the poultry industry has advanced remarkably due to improvements in bird genetics, nutritional advancements, and increased labor efficiency (Brannan and Anderson, 2021). Moving away from intensive (cage) towards extensive (cage-free) production systems requires a substantial increase in labor hours, particularly when transitioning from conventional cage to cage-free production, with an estimated 45% increase in human labor hours (Anderson, 2014). Increased labor is needed for transitioning pullets to layer housing because they must be trained to find feed and water, improving litter quality within the system towards the end of the laying cycle, and frequent inspections to minimize floor eggs, feather pecking, piling, and equipment assessment (Brannan and Anderson, 2021; de Haas et al., n.d).

Laying hens, like other poultry species, possess 4 cone photoreceptors to detect blue, green, red, and ultraviolet (UV) light that provides tetrachromatic vision (Burkhardt, 1982; Wilby et al., 2015; Seifert et al., 2020). When studying individual cone sensitivity in chickens, Wilby et al., (2015) found violet and blue cones had greater sensitivity compared to red and green cones.

UV light is composed of the shorter wavelengths (100-400 nm) of electromagnetic radiation spectrum and is divided into three parts: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm) (Rana and Campbell, 2021). UV light (UVA and UVB) may be used as a management tool to improve the welfare and health of poultry species, whereas UVC wavelengths may be utilized to protect poultry species against airborne viral infections, although

continuous exposure can cause eye damage (Lewis and Gous, 2009; Rana and Campbell, 2021). However, more research must be conducted to find "optimal timing of exposure commercially, particularly UVB wavelengths which can cause damage with high exposure and thus may limit practical application" (Rana and Campbell, 2021). During a preference test, laying hens that were 16 to 24 wks of age preferred daylight (combination of UVA and LED white light) and forest light (combination of UVA and red, green, and blue LED light) compared to conventional light (LED white) used in poultry housing and had higher frequencies of locomotion, standing and foraging behaviors (Wichman et al., 2021). Additionally, researchers assured light intensity was similar across treatments by taking measurements with a light meter at 5 different locations in study pens at bird level (Wichman et al., 2021).

An essential management practice for producers is implementing the right lighting programs throughout the production cycle of poultry species (Patel et al., 2016). For this reason, this area is of particular interest to researchers as there is an economic benefit to producers since feed consumption and growth rate can be influenced by light. Researchers have attempted to improve feed efficiency and growth rate by the addition of light flashes throughout the production cycle of broilers and dual-purpose breeds found in Egypt (Farghly, 2014; Fargly and Makled 2015). Fargly et al., (2017), investigated the effect of applying light flashes (20 pulses/min) during rearing of 360 naked neck (Sharkasi) birds (growing and laying periods). Concluding points from the study were that birds subjected to 10, 20, or 30-min of light flashes per hour had an overall better growth performance, egg production, shell thickness, and livability as opposed to birds reared under common light (12-h during growing and 16-h during laying), 40-min and 50-min of light flashes per minute (Farghly et al., 2017).

Mass depopulation of livestock and poultry is implemented under urgent circumstances, such as immediate disease control, and response to natural or human-made disasters (AVMA, 2019). The depopulation methods widely used for depopulation of poultry species include water-based foam generators or nozzles; whole house, partial house, or containerized gassing; and under constrained circumstances, ventilation shutdown plus (VSD+) (AVMA, 2019; AAAP, 2021). The use of water-based foam or gas may not be feasible at times due to the amount of area that must be treated since cost and logistics are directly proportional to the area (AVMA, 2019). Moreover, logistical problems for cage-free systems, particularly aviary systems, arise during the catching process of depopulation. Since aviary systems encourage hens to freely move through

the system, hens may be scattered throughout and require human labor for individual hens to be chased and caught (Knowles and Wilkins 1998; Gerpe et al., 2021). Another problem personnel may face is access to the hens inside of aviary systems, since catching can cause unintended injury to the hens that attempt to escape and crash into housing equipment (Knowles and Wilkins 1998; Gerpe et al., 2021).

While research has investigated effects of UV light supplementation on avian health and welfare, no research has been conducted using flashes of UV light as a management tool to move birds (Maddocks et al., 2001; Schutkowski et al., 2013; Rana and Campbell, 2021). Being able to concentrate all birds in one area may provide an appropriate method to improve flock inspections and catching hens for depopulation procedures. Therefore, the objective of this study was to determine if flashing UV light for 10-sec (2 pulse/d, 1 pulse in AM and 1 pulse in PM), combined with darkening of floor area, could elicit a movement response from laying hens reared in a cage free system. We hypothesized that when UV lights were flashed for 10-sec, hens would leave the aviary and move to the floor area, having a greater number of hens on the floor area compared to the aviary system.

## MATERIALS AND METHODS

This study was conducted at Michigan State University's Poultry Teaching and Research Center (East Lansing, MI). All procedures involving live birds were approved by Michigan State University Institutional Animal Care and Use Committee (IACUC-202100026).

### *Housing and Birds*

A total of 1,800 Lohmann Brown hens were obtained as day-old-chicks from Hy-line hatchery (Hy-line North America, LLC, GA) and transported to the Poultry Teaching and Research Center. The study began when hens were 56 wk of age and was conducted in four identical rooms measuring 19.81m x 4.57m x 3.20m (length x width x height). All rooms had an equal number of hens per aviary section. Hens were randomly divided into each room (450 hens/room) and were further divided into 3 sections (150 hens/section; 0.093 $m^2$ per bird) of the multitier aviary system (NATURA60, Big Dutchman Inc., Holland, MI). The aviary system was internally divided into 4 sections and had approximately 120 to 130 hens/section; hen housing

capacity of system per section was 144 hens/section. To mimic a commercial stocking density, hens from fourth section were divided and placed into remaining 3 sections to obtain approximately 150 hens/section (United Egg Producers, 2017).

The aviary was located in middle of each room, with each aviary section facing the wall; each section was of equal size and equipment (i.e., each section has 3 tiers, water lines, feeders, etc.), refer to **Figure 2.1**. The first tier included a water line, feeders, outer perch, and main opening to floor area; second tier included feeders and outer perch; and third tier included one water line, nest boxes, and inner and outer perches. Within each section, there was an open litter area in front of the aviary and litter area underneath the system. Birds were able to freely move within their respective sections but could not access another section of the aviary. For a detailed description of the aviary offered to birds, please see Ali et al., (2016). Hens were provided with ad libitum access to water, fed 3 times a day (with 2 stimulations), and were under a lighting schedule of 16L:8D; room lights were turned on at 0800 (dawn).

Doors on the system that restricted access to the floor area during the night (dark period) were opened prior to the start of study and kept open for the full duration of study. However, there was an equipment malfunction in the control room and one of the rail doors in tier 3 (nest boxes) remained closed. Although a portion remained closed, hens still had access to the floor by using main opening to floor area located in tier 1.

### *Lighting Treatments and Light Intensity*

Due to room setup and takedown, the experiment was conducted over the span of 4 d (1 room/d; 1 treatment/d). Each room was randomly assigned to a lighting treatment: 1) control (C), UV light flash and/or darkened floor area were not utilized, 2) UV lights flashed for one 10-sec per morning/afternoon (UV), 3) floors of the system were darkened plus UV lights flashed for one 10-sec per morning/afternoon (DF+UV), and 4) floors of the system were darkened (DF). The floor areas were darkened by turning off the floor lights on the controller, causing the floor area and tier 1 to be darker compared to the rest of the system (**Figure 2.2**). Treatments were applied once in the morning (AM) between 8 am and 9:30 am and once in the afternoon (PM) between 12 pm and 1 pm each day to observe if diurnal rhythm would influence treatment response. For the DF+UV treatment, floors were darkened 5 to 10-min prior to the UV flash. In the DF treatment, after the AM application, floor lights were turned on 60-min prior to PM

application. Since in treatment C nothing was applied (i.e., no utilization of UV light flash and/or darkened floor area), bird distribution and behavior at times when other treatments were applied were used for comparison. Two rooms were equipped with 6 UV-LED purple blacklight bars (INWT504014650Dc, Barrina Lighting, Paris, France) in the UVA light spectrum (395-400nm). UV lights were positioned on the back side of the system to encourage hens to vacate the aviary through the opened doors and onto the floor. When floor lights in UV treatment remained on, UV flash was partially observed in tier 1 whereas in tier 2 and 3 (nest box) UV flash was more noticeable. Additionally, in treatment DF+UV, the UV lights illuminated all 3 tiers. The hens did not have prior exposure to UV light.

To know whether light intensity was similar across treatments, it was measured at 3 locations at approximately bird level in each aviary section (middle of floor area, middle of tier 1, and middle of tier 2) using a light meter (cal-LIGHT 400, The Cooke Corp., San Diego, CA).

***Video Data Collection, Spatial Distribution, and Behavior Analysis***

Dome CCTV cameras with 4k ultra-HD resolution (LNE9292B, LOREX Corp., Linthicum, MD) and an NVR recording system (N882A63B, LOREX Corp., Linthicum, MD) were used, with 4 cameras per section, for 12 total cameras per room. Two cameras were mounted on the wall to record each tier of the aviary, one mounted on the ceiling for an aerial view of the floor area, and one on the backside of the aviary to record tier 1 and floor area underneath system. This set up enabled video recordings of almost the entire housing system, visibility was reduced in some areas due to space limitations of aviary equipment.

To determine the spatial distribution of hens, video recordings were observed by one observer at 1-min intervals during a 12-min observation period (6-min pre-treatment and 6-min post-treatment) per camera. Hens observed within the system and floor area were counted at each time point. In order to be counted, hens had to be clearly visible in the video frame. Then, the difference of hens for floor area was calculated by subtracting the number of hens pre- and post-treatment application.

Hen behavior was observed to determine if treatments altered behavior. The behavior categories were preening, dust bathing, wing flapping, perching, and standing alert (**Table 2.1**). Standing alert was considered a stress-related behavior (Campler et al., 2009; Bhania and Bahndauria, 2018). Perching behavior is a normal behavior observed in laying hens (Dİkmen,

2014), but was considered a stress-related behavior in this study since it provides a place of refuge from aggressors or stressful situations (Yan et al., 2014; Hartcher and Jones, 2017). Preening, dust bathing and wing flapping were considered natural comfort behaviors (Zimmerman et al., 2011; Bhanja and Bhandauria, 2018). Behavior data was collected via instantaneous scan sampling. Video recordings were observed by one observer with an observation period of 5-min pre-treatment and a second observation period of 10-min post-treatment, totaling a 15-min observation block per camera. The 5-min pre-treatment observation period was divided into 20 sets of 15-sec intervals and 40 sets of 15-sec intervals for the 10-min post-treatment application.

### *Statistical Analysis*

Data analyses were carried out using the GLM procedure of SPSS 27 (IBM, Armonk, NY). The study was a completely randomized design, and the experimental unit was an aviary section (N=3 aviary sections/room). Data were examined for normality and analyzed for interaction between treatment, position in aviary, and main treatment effects, distribution time of day, time post treatment. Statistical significance was considered at $P \leq 0.05$. Means were separated with Tukey's Least Significant Difference Test.

## RESULTS AND DISCUSSION

### *Spatial Distribution of Hens*

Spatial distribution results are presented as means ± standard error of the mean (S.E.M). No difference was detected in the spatial distribution of hens before treatments were applied. For the AM application, at min 0 (i.e., immediate time period that followed treatment application), there was a significant difference ($P$=0.001) between treatments, with DF+UV treatment having the highest difference of hens in floor area (i.e., more hens in the floor area after treatment application) compared to the other treatments (**Table 2.2**). At min 1, C, DF, and UV treatments were comparable between one another but had a lower difference (P<0.001) than DF+UV treatment. Min 2, 3, 4, and 5 followed a similar trend to min 1, meaning C, DF, and UV treatments had a mean comparable between one another but significantly lower than DF+UV ($P$<0.001; $P$=0.005 in min 5). DF+UV treatment had the overall highest difference of hens

located in floor area at each timepoint when compared to the other treatments, with highest occurrence at minute 2 (**Table 2.2**).

For the PM application, at min 0 DF+UV treatment had a higher difference of hens located in floor area (*P*=0.017) than C treatment; DF and UV treatments had a similar difference of hens when compared individually to C and DF+UV treatments (**Table 2.3**). Min 1, 2 and 4 followed a similar trend from min 0, meaning that a lower difference (P=0.025 in min 1; P=0.044 in min 2; P=0.021 in min 4) was detected in C treatment when compared to DF+UV treatment, but DF and UV treatments had a difference of hens similar to C and DF+UV treatments when compared individually. At min 3 and 5 no significant differences were detected between treatments (P=0.103 in min 3; P=0.109 in min 5). In PM application, the DF+UV treatment had an overall higher difference of hens located floor area when compared to C treatment, but was the same compared to other treatments, with exception of minute 3 and 5 (**Table 2.3**).

Based on the location of UV lights (i.e., to stimulate hens' movement towards the floor area) the UV light may had been perceived as an environmental stressor that was followed by a fleeing response from hens' movement to the floor area to escape the UV light. There may be 2 possible explanations as to why hens reacted in such a manner. When an animal encounters a short-term challenge (i.e., predation attempt or change to the immediate environment) "the physiological stress response, coordinated by the activation of the hypothalamic-pituitary-adrenal (HPA) axis, provides an essential mechanism for survival designed to help the animal escape the stressor and return to stable conditions" (Pusch et al., 2018). The first explanation is related to the personality of brown hens (i.e., proactive personality) (Cockrem, 2013; Pusch et al., 2018). Personality is defined here as "birds' response to changes in their immediate environment that is consistent with individual behavioral and physiological responses" (Cockrem, 2013). Proactive animals tend to have a bold and fast response to unfamiliar environments or stimuli (i.e., quick explorers, less fearful and more aggressive) and produce smaller physiological responses (e.g., corticosterone elevations) to acute stressors (Cockrem, 2013; Pusch et al., 2018).

A second explanation for the hens' reaction could be related to the flicker rate of the UV lights. While a statement can be found in the manual of UV lights used, stating that these lights do not flicker, and no flickering was detected by the naked human eye, the avian eye can perceive rapid movements (high flicker fusion frequency) (Rubene et al., 2010; Korbel, 2012; Lisney et al., 2012). Flicker fusion frequency is defined as "the rate of successive light flashes

from a stationary light source at which the sensation of flicker disappears, and the light becomes 'steady'" (Simonson and Brozek, 1952). In the study by Lisney et al., (2012) the retina of adult laying hens was examined, via electroretinograms, to determine whether the chicken retina can detect flicker at higher frequencies. The results showed the retina of hens can at least respond to flicker frequencies in the 100 to 200 Hz range, although Lisney et al., 2011, found that hens do not appear to be able to consciously detect flicker above approximately 90 Hz. The retina's ability to respond to flicker frequencies higher than 100 Hz may result in distress for the animals (Lisney et al., 2012). Although a light meter was not used to determine if flickering was apparent in the UV lights, there was a quick and effective way this was resolved by, using the slow-motion video setting (240 frames per second) in a standard iPhone. While the frequency of the flicker cannot be determined with this procedure, flickering can be detected and indeed, the UV light did flicker. Therefore, flickering may have caused hens to experience general stress and move to the floor area away from UV light (Greenwood et al., 2004; Lisney et al., 2011).

A second plausible explanation, related to light flickering, may give insight as to why hens reacted to such an extent. Rubene et al., (2010) concluded that when some UV light is added to regular white light, birds can detect higher frequencies of flickering light, compared to only white light, meaning that the temporal resolution was enhanced by the addition of UV wavelengths. Since hens in the present study had no prior exposure to UV light, it may be plausible that the UV light flash caused some distress due to improved temporal resolution during those 10-sec.

When examining the mean difference of hens in floor area between AM and PM application (**Table 2.2 and Table 2.3**, respectively), it is evident that treatments had a stronger effect on hens in the AM application than PM application. A potential justification for this is that in AM application, treatments were novel to hens as they did not have prior exposure to UV lights. In a study conducted by Jones (1977), 909 male chicks were separated into groups that were presented with different rearing cues (red crosses or black circles) on the walls of their home environment from 2 to 7 d old; behaviors were measured during an open field test in absence or presence of familiar rearing cues. After chicks reached 7 d of age, they were tested individually in a test box; the researcher found fearfulness (freezing, sitting time, lying time, and time spent with eyes closed) was reduced in the presence of familiar rearing cue, meaning that familiarity decreased fearfulness (Zajonc et al., 1974; Jones, 1977). An interesting finding was

that when chicks were in the presence of their familiar cue, they spent more time drinking and eating, which could potentially support notion that chicks were less fearful (Jones, 1977). Campbell et al., (2016a) studied hen pattern movements between the aviary system and floor area at various times (morning, afternoon, and evening) throughout 2 different flock cycles (peak lay, mid lay, and end of lay). For both flocks, more hens per unit moved from the system onto floor area in the morning period as soon as hens gained access to it; but a higher occupancy of the floor area was more apparent and consistent during afternoon period (Campbell et al., 2016a).

***Light Intensity***

Mean light intensity (lx) in rooms post-treatment application can be found in **Table 2.4**. When comparing light intensity between treatments for floor area, there were no differences ($P \geq 0.05$) detected. In tier 1, there were differences ($P \leq 0.05$) in light intensity detected between all treatments, with DF treatment having the lowest light intensity (1.4 ± 0.03 lx) while UV treatment had the highest (7.7 ± 0.4 lx). For tier 2, non-UV light treatments had the same light intensity (C=3.2 ± 0.2 lx; DF= 3.3 ± 0.1 lx) but differed ($P \leq 0.05$) from treatments that utilized UV lights (UV= 8.9 ± 0.3 lx; DF+UV= 8.5 ± 0.3 lx). According to Hy-line (2017) technical bulletin published on poultry lighting for egg producers, light intensity (measured in lux, clux or food candles (fc)) is essential for poultry production. High light intensity (above 50 lx) "may cause nervousness and aberrant behavior, while light intensity below 5 lx is too dark to stimulate proper growth and production" (Hy-line 2017). For laying hens that have been transferred to layer house, an average of 30 lx at the level of feed trough is recommended (Hy-line 2017). This recommendation differs from broiler production (Aviagen 2018). Deep et al., (2013) investigated what was the required minimum standard light intensity needed for optimal production and welfare of broilers, the light intensity explored during study were 0.1, 0.5, 1, 5, and 10 lx (broilers were exposed to 40 lx for first 7 d of age before abruptly switching to treatments). Authors concluded that 0.1 lx was inadequate as it led to lower feed intake and overall higher mortality compared to birds exposed to 1,0.5, 5, and 10 lx, whereas 0.5 to 10 lx did not differ on broiler mortality over treatment period (Deep et al., 2013). Additionally, authors recommended that at least 5 lx be maintained for light intensity for optimal production and welfare, while anything less than 1 lx reduces productivity and welfare (Deep et al., 2013). Based on findings

from present study, it can be inferred that the light intensity in treatment rooms, particularly in aviary tiers, was low and not high, meaning light intensity was not high enough to cause aberrant behaviors.

Furthermore, observations of hens' reaction after UV flash were used revealed that hens who had their backs to the UV light, or had their heads in feed trough, did not react to UV light in the same manner as hens who were in direct contact (i.e., fleeing response). Instead, those hens stayed in aviary system looking around being vigilant.

***Behavioral Data Collected from Hens***

The results for behavioral observations after treatments were applied are shown in **Table 2.5**. C treatment had, overall, the highest frequency ($P$=0.01) of preening behavior when compared to UV treatment and DF+UV treatment but comparable expression to DF treatment. Hens from both UV light treatments had a lower frequency of preening. For perching behavior, there was a significant difference ($P \leq 0.001$) between the DF treatment and other treatments, with DF having the lowest frequency of perching. DF and DF+UV treatments were significantly different ($P$=0.007) in frequency of dust bathing behavior between one another, but when each is compared to C and UV treatments, they are comparable in frequency. For wing flapping, C and DF treatments were similar but had higher frequency ($P \leq 0.001$) compared to UV and DF+UV treatments. Standing alert was highest in UV treatment and DF+UV treatment when compared to C and DF treatments ($P \leq 0.001$).

Overall, UV light influenced the behavior of hens as both groups in rooms with UV lights had lower frequencies of preening and wing flapping (comfort behaviors) but had a higher expression of standing alert (stress behavior), whereas more hens exhibited comfort behaviors in the non-UV light treatments. Interestingly, an increase in preening (comfort behavior) expression was anticipated after UV light utilization as disruption to hen environment occurred (i.e., stressful situation), this occurrence is referred to as displacement behavior. Displacement behaviors such as preening, head shaking or vocalization, are behaviors birds will do to show signs of frustration, stress, and overall discomfort (Duncan and Wood-Gush, 1972; Kuhne et al., 2013). Results from present study demonstrated that although hens in UV treatments exhibited signs of stress (i.e., higher standing alert frequency), hens were still comfortable in the environment. Moreover, Zimmerman et al., (2011) conducted a study in 17-wk old hens to find

behavioral expressions specific to the anticipation of a positive, neutral, and negative event. The authors observed that anticipation of a positive event was associated with an increase in comfort behaviors (preening, wing flapping, feather ruffling and body scratching), while hens in the negative event showed more head movements and higher locomotion. Although hens in the present study were not anticipating a positive or negative event, it could be inferred that natural comfort behaviors are expressed to reflect how well the animal is feeling in the current environment and why lower frequencies of these behaviors were lowest in both UV treatments (Linares and Martin, 2010; Bhanja and Bhadauria, 2018).

Domestication may have played a role in hens displaying a higher frequency of standing alert behavior. Domestication is defined as a process by which a population of animals becomes adapted to a captive environment by the combination of genetic changes and environmentally induced developmental events (Price, 2002). The Red Junglefowl is considered the wild ancestor of domestic chickens (Fumihito et al., 1994; Hartcher and Jones, 2017; Tixier-Boichard, 2020). In a study conducted by Campler et al., (2009) domesticated White Leghorn chickens and Red Junglefowl were tested for behavioral reactions to 4 different types of potentially fearful stimuli. Results indicated Red Junglefowl had higher fear levels than White Leghorn across the 4 fear tests and performed more stand/sit alert and less locomotion, fly/jump, and vocalizations. Whether it was a conscious or unconscious decision to select for less fearfulness in chickens, domestication contributes to this phenomenon as indicated by the results presented. However, molecular studies show that a consequence of intensive selection is the loss of genetic diversity at the DNA level (Tixier-Boichard, 2020).

Overall results indicated that a flash of UV light combined with darkened floors (DF+UV treatment) was the most effective in moving hens out of an aviary system and onto floor area. However, the treatment used might not be effective for long term movement since hen concentration varied within a 6-min observation period.

## CONCLUSIONS AND APPLICATIONS

1. Utilization of UV lights coupled with darken floors had a stronger reaction on hens, compared to C, UV, and DF treatments. However, this reaction was observed when DF+UV treatment was novel in AM application and not when it was applied in PM application.
2. Although the UV light flash caused hens to concentrate in floor area, the technique of UV flash of 10-sec might not be feasible during mass depopulation procedures as hens did not stay concentrated for longer than 6-min.
3. Behavioral differences were seen among the 4 lighting treatments with more comfort behaviors (preening and wing flapping) expressed in non-UV treatments when compared to treatments that utilized UV light.
4. Future research should focus on usage of UV lights in preventing undesirable behaviors (i.e., floor eggs and piling) since this technique provoked an immediate reaction from hens. Piling behavior is a common concern in laying hens because it can lead to decreased welfare and productivity; yet this is not a concern in younger chicks since it can aid in thermoregulation (Bright and Johnson, 2011; Campbell et al., 2016b; Gray et al., 2020). However, the consequences for long-term use of UV-light flash are not known and should be further explored.

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**Table 2.1.** Ethogram of the 5 behaviors evaluated during the observation period.

| Behavior Category | Behavior | Description |
|---|---|---|
| **Positive- comfort** | Preening[1,2] | Bird uses beak to groom feathers on different regions of the body; sometimes uses oil from uropygial gland to groom feathers. |
| | Dust bathing[2] | Bird may scratch or squat on the litter, uses different body movements to force the litter to coat the feathers. |
| | Wing flapping[1] | Bird is in upright position, extending its wings in fast, repeated movements. |
| **Negative- stress** | Standing alert[2,3] | Bird is in upright position, with the neck extended upright |
| | Perching[4,5] | Bird holds onto the perch with feet. May be seen in an upright position or breast may be in contact with the perch. |

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**Table 2.2.** Mean difference of the number of hens (pre- and post- treatment application) located in floor area in AM application (6-min).

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| **Min** | **Control** | **DF** | **UV** | **DF+UV** | ***P*-value[2]** |
| 0 | $5.3^{a} \pm 0.7$ | $27.0^{ab} \pm 4.9$ | $37.3^{b} \pm 2.4$ | $71.7^{c} \pm 13.0$ | 0.001 |
| 1 | $10.0^{a} \pm 3.2$ | $23.0^{a} \pm 4.0$ | $38.0^{a} \pm 9.2$ | $71.3^{b} \pm 7.2$ | <0.001 |
| 2 | $11.0^{a} \pm 1.5$ | $20.3^{a} \pm 6.4$ | $33.0^{a} \pm 10.8$ | $102.7^{b} \pm 14.2$ | <0.001 |
| 3 | $8.7^{a} \pm 0.7$ | $13.0^{a} \pm 3.5$ | $33.0^{a} \pm 6.2$ | $75.0^{b} \pm 10.3$ | <0.001 |
| 4 | $11.7^{a} \pm 4.6$ | $15.3^{a} \pm 5.5$ | $30.3^{a} \pm 3.7$ | $78.3^{b} \pm 10.7$ | <0.001 |
| 5 | $13.0^{a} \pm 3.6$ | $21.7^{a} \pm 6.1$ | $27.0^{a} \pm 7.0$ | $67.3^{b} \pm 11.9$ | 0.005 |

[1] Control=control, nothing was applied; DF=the floor of the system and tier 1 were darkened; UV= UV lights turned on for 10-sec; DF+UV=the floor of the system was darkened plus UV lights turned on for 10-sec.
[2] Means within a row lacking a common superscript differ ($P \leq 0.05$).

**Table 2.3.** Mean difference of the number of hens (pre- and post-treatment application) located in floor area in PM application (6-min).

| | **Treatments**[1] | | | | |
|---|---|---|---|---|---|
| **Minute** | **Control** | **DF** | **UV** | **DF+UV** | ***P*-value**[2] |
| 0 | $12.0^{a} \pm 1.2$ | $22.0^{ab} \pm 5.0$ | $31.0^{ab} \pm 8.3$ | $55.3^{b} \pm 11.1$ | 0.017 |
| 1 | $9.3^{a} \pm 2.9$ | $23.7^{ab} \pm 5.7$ | $31.0^{ab} \pm 7.5$ | $53.0^{b} \pm 12.1$ | 0.025 |
| 2 | $11.3^{a} \pm 1.7$ | $26.7^{ab} \pm 7.1$ | $29.7^{ab} \pm 10.4$ | $47.3^{b} \pm 6.6$ | 0.044 |
| 3 | $15.3 \pm 4.7$ | $20.0 \pm 4.0$ | $25.0 \pm 8.4$ | $37.0 \pm 3.5$ | 0.103 |
| 4 | $9.3^{a} \pm 0.9$ | $29.0^{ab} \pm 3.8$ | $33.3^{ab} \pm 7.8$ | $48.3^{b} \pm 10.2$ | 0.021 |
| 5 | $17.3 \pm 2.4$ | $24.0 \pm 2.1$ | $39.7 \pm 10.3$ | $43.0 \pm 10.0$ | 0.109 |

[1] Control=control, nothing was applied; DF=the floor of the system and tier 1 were darkened; UV= UV lights turned on for 10-sec; DF+UV=the floor of the system was darkened plus UV lights turned on for 10-sec.
[2] Means within a row lacking a common superscript differ ($P \leq 0.05$).

**Table 2.4.** Mean light intensity in the treatment rooms.

| | Light Intensity (lx) | | |
|---|---|---|---|
| **Treatment** | **Floor area[1]** | **Tier 1** | **Tier 2** |
| Control | 35.1 ± 0.4 | $2.9^{b}$ ± 0.07 | $3.2^{a}$ ± 0.2 |
| DF | 34.5 ± 0.2 | $1.4^{a}$ ± 0.03 | $3.3^{a}$ ± 0.1 |
| UV | 34.9 ± 1.0 | $7.7^{d}$ ± 0.4 | $8.9^{b}$ ± 0.3 |
| DF+UV | 35.2 ± 0.4 | $5.2^{c}$ ± 0.3 | $8.5^{b}$ ± 0.3 |

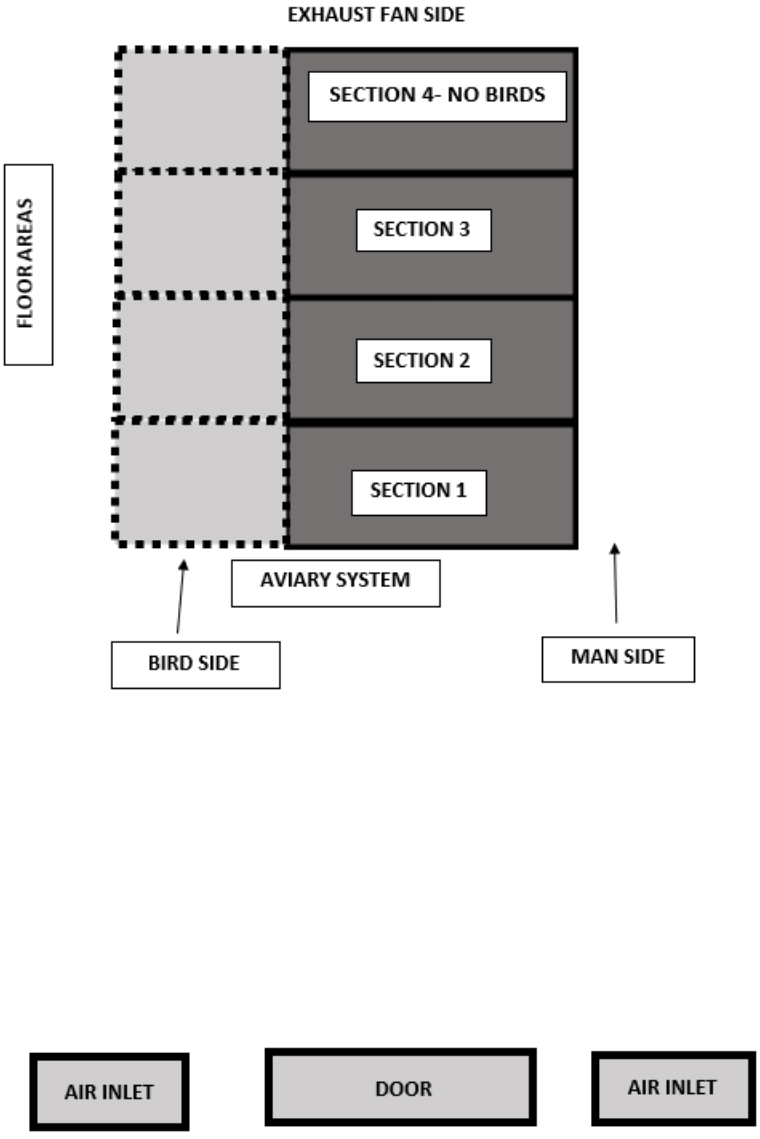
[1] Means within a column lacking a common superscript differ ($P \leq 0.05$).

**Table 2.5.** Mean frequency of occurrence for behavioral observation.

| | **Behaviors** | | | | |
|---|---|---|---|---|---|
| **Treatments** | **Preening** | **Perching** | **Dust Bathing** | **Wing Flapping** | **Standing Alert** |
| Control | $0.33^{c} \pm 0.020$ | $0.23^{b} \pm 0.017$ | $0.02^{ab} \pm 0.006$ | $0.12^{c} \pm 0.009$ | $0.08^{a} \pm 0.013$ |
| UV | $0.23^{a} \pm 0.016$ | $0.22^{b} \pm 0.012$ | $0.02^{ab} \pm 0.006$ | $0.09^{ab} \pm 0.005$ | $0.20^{b} \pm 0.016$ |
| DF | $0.29^{bc} \pm 0.010$ | $0.14^{a} \pm 0.007$ | $0.04^{b} \pm 0.008$ | $0.11^{bc} \pm 0.007$ | $0.10^{a} \pm 0.009$ |
| DF + UV | $0.25^{ab} \pm 0.016$ | $0.24^{b} \pm 0.011$ | $0.01^{a} \pm 0.004$ | $0.08^{a} \pm 0.005$ | $0.21^{b} \pm 0.016$ |
| *P*-value[1] | 0.01 | <0.001 | 0.007 | <0.001 | <0.001 |

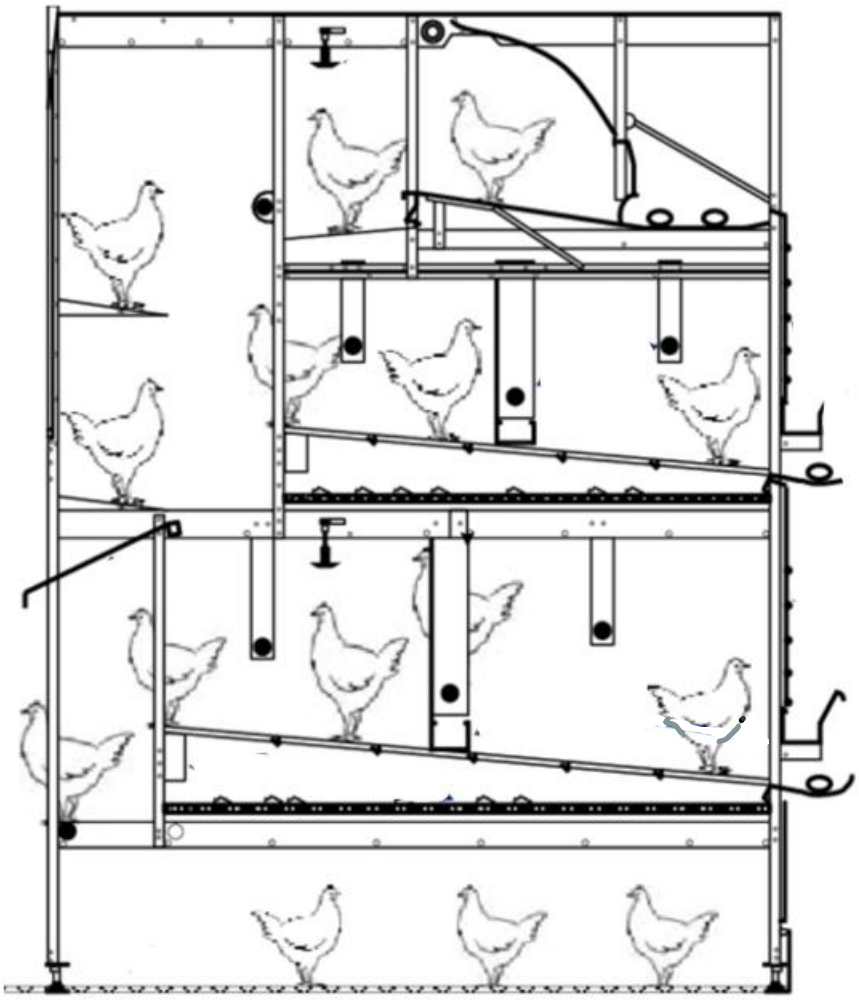
[1] Means within a column lacking a common superscript differ ($P \leq 0.05$).

**Figure 2.1.** Aerial view of room layout.




Aerial view of room layout. Room layout was identical between the treatments. Aviary system was in the middle of room and consisted of 4 sections, each with corresponding floor area; however, only 3 sections were used to house hens in present study. Bird side depicts where hens were located. Man side represents area workers used for egg collection and side where UV lights were placed. Front end of room contained entrance door and 2 air inlets, whereas 2 exhaust fans were located on back end of room. Diagram not drawn to scale.

**Figure 2.2.** Aviary system offered to hens.



Aviary system offered to hens. The levels of the aviary (from bottom to top) are floor, tier 1, tier 2, and tier 3 (nest boxes). UV lights were placed right above feed trough. Source: Zhao, Yang & Zhao, Deiling & Xin, Hongwei. 2013. Characterizing Manure and Litter Properties and Their Carbon Dioxide Production in an Aviary Laying-Hen Housing System. American Society of Agricultural and Biological Engineers Annual International Meeting 2013, ASABE 2013. 4. 10.13031/aim.20131618601.

# CHAPTER 3

## CAN STEAM BE USABLE AS A "PLUS" FOR VENTILATION SHUTDOWN?

**Submitted for publication to Poultry Journal from MDPI**

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## ABSTRACT

The American Veterinary Medical Association (AVMA), preferred depopulation methods (i.e., foam, containerized gassing, and mechanical methods) can be challenging when depopulating commercial laying hen barns for multiple reasons. When preferred methods are not feasible, ventilation shutdown plus (VSD+) heat and/or carbon dioxide ($CO_2$) is approved for emergency situations. Both "plusses" work but can have issues as VSD+ heat typically causes a decrease in environmental humidity and can affect plastic structures, while $CO_2$ can be a human safety concern and procurement problem during emergencies. Steam supplies both heat and humidity, the latter hindering birds' ability to dissipate body heat through evaporative cooling, thus expediting hyperthermia. The objective of this study was to evaluate effectiveness of VSD+ with steam as a "plus" for depopulation of laying hens in a cage-free aviary system. Approximately 1,800 Lohmann Brown hens aged 56 weeks were housed in Big Dutchman Natura 60 aviaries in four rooms. Four VSD+ treatments were as follows: 1) control or VSD+ heat (VSD-H), 2) VSD with steam (VSD-S), 3) VSD with heat and then steam to maintain temperature (VSD-HS), and 4) VSD with steam and then heat to maintain temperature (VSD-SH). All VSD+ procedures followed AVMA depopulation guidelines for temperature and time (i.e., 40°C within 30 minutes). Hens were monitored via cameras for time to first and 100% mortality. After depopulation was completed mortality location within each tier of system (floor area, 1st tier, 2nd tier and nest boxes) were recorded. Data were analyzed in SPSS v. 28 and significance was at $P<0.05$. Observed time to first mortality for VSD-H, VSD-S, VSD-HS, and VSD-SH were 82.7-min, 56.6-min, 49.6-min, and 52-min. While for 100% mortality for VSD-H was 180-min+ (never reached 100% mortality in time limit); VSD-S was 112.3-min; VSD-HS was 83.3-min; and VSD-SH was 103.6-min. Location of mortality revealed VSD-S and VSD-SH had more carcasses located in floor area than VSD-HS ($P=0.02$); 1st tier and nest boxes tier VSD-S and VSD-SH had less carcasses than VSD-HS ($P=0.02$); no differences were observed between treatments in 2nd tier ($P=0.248$). Hens in steam treatments were faster in reaching time to first and 100% mortality than hens in VSD-H ($P<0.05$). Results indicated that steam alone, or in combination with forced air heat, could be used as a "plus" for VSD+ depopulation of laying hens reared in a cage-free or aviary housing system.

## INTRODUCTION

Over the last decade, several events have led to emergency mass depopulation of domestic poultry. In 2015, the highly pathogenic avian influenza (HPAI) outbreak resulted in depopulation of 7.5 million turkeys and 42.1 million egg-layer and pullet type chickens (USDA-APHIS, 2016a). During the Covid-19 pandemic, some processing plants ceased operation due to disease outbreaks amongst workers, and premises cleaning and disinfection for workers to safely return (Marchant-Forde and Boyle, 2020). In addition, decreased demand during Covid-19 for poultry products from restaurants and foodservice businesses resulted in the need for less poultry to be processed (Sharma et al., 2020). Decreased demand and processing plant closures resulted in emergency depopulation of 2 million broilers and 61,000 laying hens (Kevany, 2020). Beginning in 2022, the United States of America (US) experienced another extensive HPAI outbreak. At time of writing, close to 60 million domesticated fowl have been depopulated (including both commercial and backyard flocks) (USDA-APHIS, 2022). According to the US Department of Agriculture, Economic Research Service (USDA-ERS), a total of 43.3 million table-egg layers were lost to HPAI in 2022 (USDA-ERS, 2023).

In 2019, the American Veterinary Medical Association (AVMA) released guidelines for depopulation of livestock and poultry; approved methods fall under two categories, “preferred” and “acceptable under constrained circumstances”, but ultimately USDA has final decision on what methods will be used (USDA-APHIS, 2017). For poultry, methods are approved based on whether birds are indoors or outdoors and if birds are floor-reared or caged. For present study, the focus will be on floor-reared confined poultry, as aviary style housing was included in this category (AVMA, 2019; Marchant-Forde and Boyle, 2020). Preferred depopulation methods for this category are water-based foam; whole house, partial house, or containerized gassing; cervical dislocation, mechanical assisted cervical dislocation; and captive bolt gun (AVMA, 2019; AAAP, 2021). Additionally, acceptable methods under constrained circumstances are: VSD+, controlled demolition, exsanguination, and decapitation (AVMA, 2019; AAAP, 2021). When a farm has tested positive for HPAI, USDA Animal and Plant Health Inspection Service (APHIS) guidelines require initiation of depopulation within 24 to 48-h to avoid prolonged suffering of birds and “stamp out” virus (USDA-APHIS, 2016b). However, such time constraints may not allow adequate preparation time for implementation of AVMA’s preferred depopulation methods. In such cases producers, alongside government response officials, must consider

methods approved under constrained circumstances, most notably ventilation shutdown plus heat and/or $CO_2$ (VSD+).

VSD+ has been approved as a last resort method if preferred methods do not meet required USDA foreign animal disease response timing criteria due to a lack of available resources, worker safety or logistics (Gingerich, 2015).VSD is defined as the "cessation of natural or mechanical ventilation of atmospheric air in a building where birds are housed, with or without action to increase ambient temperature, resulting in an increase of indoor temperature and eventual death of animals" (Gingerich, 2015). However, VSD alone is not recommended under any circumstances and supplemental heat and/or carbon dioxide ($CO_2$) must be added, referred to as VSD+.

Eberle-Krish et al., (2018), investigated addition of $CO_2$ to VSD+ to depopulate laying hens in a conventional-caged structure. One hundred percent mortality was achieved within 90-min by addition of $CO_2$ and within 120-min with supplemental heat. However, VSD alone was unable to reach 100% mortality after 225-min of implementation. Zhao et al., (2019) modeled the indoor environment and supplemental heat requirement for VSD+. Modeling and experimental results indicated that hyperthermia was leading cause of death during VSD+ and noted that having a high relative humidity would help hasten depopulation procedures (Zhao et al., 2019).

A major limitation to $CO_2$ usage is associated cost and supply shortage. Currently in the US, there is a $CO_2$ shortage (Greenwood, 2020; Taylor, 2022; Clouse, 2022; Chappell, 2022). $CO_2$ supply shortage can be attributed to contamination in largest natural $CO_2$ production hub, planned/ unplanned ammonia plant closures for maintenance, decreased fuel demand and production during Covid-19 pandemic, and labor shortages in transportation (Greenwood, 2020; Taylor, 2022; Clouse, 2022; Chappell, 2022). When evaluating $CO_2$ properties, $CO_2$ is denser than air, so a higher concentration would be found in floor area of an aviary system compared to remainder of system. Stratification of $CO_2$ becomes problematic in these systems because chickens can detect $CO_2$ concentrations at very low levels (5.0%-7.5%) and will actively choose to avoid inhaling air with 60% $CO_2$, meaning birds might move from floor area to higher tiers of aviary (Raj and Gregory, 1991; Sandilands et al., 2011).

After death, removal of carcasses from these systems could be challenging due to rigor mortis, especially leg and foot muscles that contract and "grasp" metal wire flooring in aviary

housing systems (Duncan, 2001). Elevated environmental temperatures may also influence onset of rigor mortis. Carcasses of animals that have died in a hotter environment, such as the temperatures observed in VSD+, would encounter accelerated chemical changes leading to autolysis as opposed to in a colder environment (Mesri et al., 2017).

Birds are homeothermic animals and can maintain a core body temperature between 40°C and 42°C, when environmental temperature is within thermoneutral zone (18°C to 24°C) (Donald and Williams, 2001; Anderson and Carter, 2004; Daghir, 2008; Nawab et al., 2018; Lohmann Tierzucht, n.d; University of Kentucky, n.d). There are two anatomic features that can delay birds' ability to effectively dissipate heat: feathers and absence of sweat glands. Feathers function as a form of insulation, trapping warm air close to the body, which inhibits heat loss (Donald and Williams, 2001; Anderson and Carter, 2004). Birds can use head appendages (e.g., comb) and unfeathered area under wings (i.e., apteria) to aid in direct heat loss; however, chickens use a process of respiratory evaporative cooling to compensate for absence of sweat glands (Donald and Williams, 2001; Gerken et al., 2006; Daghir, 2008). Respiratory evaporative cooling is when moisture evaporates from the damp lining of the respiratory tract (Donald and Williams, 2001; Lohmann Tierzucht, n.d; University of Kentucky, n.d). Birds will increase their respiration rate through two stages of thermal panting (referred to as thermal tachypnea or thermal polypnea) to increase respiratory evaporative cooling and reduce core body temperature (Whittow, 2000). Using thermal panting to reduce core body temperature can be a vigorous activity, especially at higher humidities, for this reason many birds supplement thermal panting with rapid fluttering of the gular area (gular flutter) (Whittow, 2000). Although respiratory evaporative cooling can be extremely effective in maintaining core body temperature, there are consequences if birds pant for a prolonged period, primarily increased loss of dissolved carbon dioxide in blood. Excessive loss of carbon dioxide results in an increased blood pH, causing respiratory alkalosis and eventual death if not resolved (Donald and Williams, 2001; Anderson and Carter, 2004; Robertshaw, 2006).

Both elevated temperature and relative humidity can have detrimental effects on birds' health if not managed properly (Donald and Williams, 2001; Daghir, 2008). For example, if ambient temperature reaches approximately 29.4°C, but relative humidity stays low (~50%), birds are still able to effectively dissipate heat through evaporative cooling (Nawab et al., 2018; Lohmann Tierzucht, n.d). However, when relative humidity increases above 70%, the amount of

moisture that can be evaporated from birds' respiratory tract decreases and therefore amount of heat that can be removed through thermal panting or gular flutter decreases (Lohmann Tierzucht, n.d). During the study performed by Eberle-Krish et al., (2018), the observed relative humidity during VSD+ procedures were between 74% and 92% for VSD; within 73% and 84% for VSD with supplemental heat; and 80% to 88.9% for VSD with addition of $CO_2$. A plausible reason as to why relative humidity was high during this study is related to how the experimental room was designed. Authors described the cages as completely enclosed from both sides, creating a chamber; the chamber was then sealed with a 10-mil polyethylene plastic ceiling (Eberle-Krish et al., 2018). Since ventilation of room was turned off, and chambers were sealed and tightly enclosed, moisture loss through hen's evaporative cooling was trapped, thus leading to a higher humidity that cannot escape. Room setup may not accurately depict relative humidity of a large commercial facility.

While forced air heaters increase temperature, air is passed through a flame or heating element and relative humidity is decreased. Utilization of steam as a "plus", should simultaneously increase temperature and relative humidity of birds' environment, consequently hindering ability to effectively dissipate heat, thus leading to a quicker depopulation process. Although steam as a "plus" has not been explored in poultry, VSD+ steam was investigated for swine depopulation. During the Covid-19 pandemic Baysinger et al., (2021) explored alternatives to depopulate swine in US Midwest region, particularly through use of VSD+ supplemental heat and steam. VSD+ steam surpassed AVMA recommendations with a 95% mortality rate in <1h and overall survival rate of 0.3%. Therefore, the objective of the current study was to evaluate the effectiveness of VSD+ with steam as a "plus" for depopulation of laying hens in a cage-free system. The hypothesis was that supplementation of steam would increase temperature and relative humidity of the laying hen room during VSD+ and expedite time to death through hyperthermia.

## MATERIALS AND METHODS

Research was conducted at Michigan State University Poultry Teaching and Research Center (East Lansing, MI). All procedures involving live birds were approved by Michigan State University Institutional Animal Care and Use Committee (IACUC-202100026).

***Housing and birds***

For this study, approximately 1,800 Lohmann Brown hens aged 56 weeks were housed at Michigan State University Poultry Teaching and Research Center (Hy-Line, n.d). Hens were kept in four identical rooms measuring 19.81m x 4.57m x 3.20m (length x width x height) that were equipped with Big Dutchman Natura 60 Aviaries (Big Dutchman, n.d). Each room had approximately 450 hens and rooms were further divided into three identical sections (150 hens/section; 0.093 $m^2$/bird) of the multitier aviary system to approximate industry stocking density (United Egg Producers, 2017). Each aviary section had identical equipment arrangement (i.e., three tiers, floor area, water lines, feeders, nest boxes). The first tier included a single water line, feeder line, outer perch, and opening to floor area; second tier had feeder line, and outer perch; and third tier included single water line, nest boxes, and inner and outer perches. Hens were able to freely move within their respective sections but could not access other sections of aviary since sections were internally divided by a wire mesh.

***Experimental room design***

The multitier aviary was located in the center of each room, with each aviary section facing the wall (**Figure 3.1**). On one side, there was a worker isle that was used for egg collection (referred to as man side), while the other side was the sections of the aviary (referred to as bird side). The front end of the room contained two air inlets and entrance to room (referred to as controller side), and the back end contained two exhaust fans (referred to as exhaust fan side). A 76.2-cm 9000 CFM pedestal fan was positioned within rooms to mitigate heat stratification (Menards, n.d-a). Immediately prior to VSD+, the drinking water system was turned off. Although nest boxes were closed before VSD+ treatments were applied, hens still had access to 3$^{rd}$ tier. There was one room (VSD-HS treatment) where nest boxes could not be closed due to equipment malfunction.

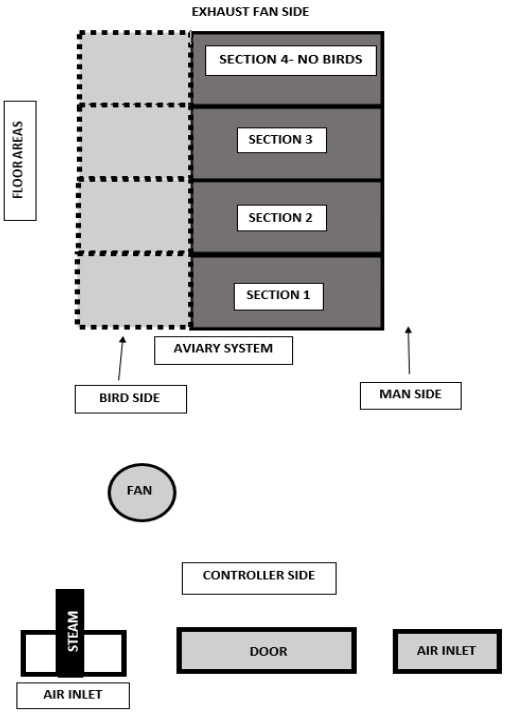


**Figure 3.1.** Aerial view of treatment room layout. Room layout and measurements were identical between treatments (19.81m x 4.57m x 3.20m (length x width x height)), with the exception for VSD-H treatment since no steam was supplemented. Aviary system consisted of four sections, each with their own floor area; however, only three sections were used to house hens in present study. Diagram not drawn to scale.

### *VSD+ treatments*

Due to daily boiler, room setup and takedown, treatments were conducted over the span of four days (1 room/day) and each room was randomly assigned to a VSD+ treatment (1 treatment/day). Temperature and relative humidity outside of the facility (where the boiler was located) were monitored by World Weather forecast service, (n.d). In accordance with AVMA standards, the goal was to raise the temperature of rooms to 40°C (104°F), within 30-min, and maintain temperature between 40°C and 43.3°C (104°F to 110°F) for a minimum of 3-h (AVMA, 2019). All data collection had a 3-h limit due to MSU IACUC protocol. Hens that remained alive past 3-h limit were humanely euthanized, either through cervical dislocation or use of $CO_2$ depopulation cart according to farm protocols. Considering that steam boiler usage did not allow for relative humidity to be manipulated to a specified percentage, two combination treatments (VSD-HS and VSD-SH) were created.

**Treatment 1: VSD with supplemental heat (VSD-H)**

In this treatment, the room ventilation system was shut off, air inlets were sealed via

wood panels and door was sealed by a fire-retardant plywood sheath (Menards, n.d-b). Only supplemental heat was added to the room via forced air heaters. The salamander forced air heater was used to heat room to 40°C, after which the wall mounted forced air heater was used to maintain temperature. If a temperature higher than 49°C was observed, the forced air heater was stopped and turned on again if temperatures decreased below 43°C.

**Treatment 2: VSD with steam (VSD-S)**

In treatment 2, the ventilation system was shut off and the room was sealed in same manner as treatment 1, but the door of the room was closed off (as opposed to usage of fire-retardant plywood sheath in treatment 1). Only supplemental steam was introduced into room through low pressure steam boiler.

**Treatment 3: VSD with supplemental heat and steam (VSD-HS)**

In treatment 3, the ventilation system was shut off and room was sealed in same manner as treatment 1. Supplemental heat and steam were added to the room. First, the salamander forced air heater heated room to 40°C. After 40°C was achieved, steam was administered to room. Treatment explored if steam could maintain the required temperature for 3-h.

**Treatment 4: VSD with supplemental steam and heat (VSD-SH)**

In treatment 4, the room ventilation system was shut off and room was sealed in same manner as treatment 2. Supplemental steam and heat were added to room. First, steam was administered to heat room to 40°C. After 40°C was achieved, steam pressure was reduced (not completely turned off) and the wall mounted forced air heater was used to maintain heat. This treatment investigated whether steam could reach the required temperature within 30-min.

***Environmental and animal monitoring***

Ambient temperature and relative humidity of rooms was recorded via USB data loggers (LASCAR Electronics, n.d). Data loggers were placed on upper and lower positions of aviary system (1$^{st}$ and 3$^{rd}$ tiers), <1.22 m vertical distance from one another, and in front and back sections of aviary to collect environmental data (4 loggers/room). Data loggers were set to record temperature and relative humidity every 30-s. The controller system from each room was used to monitor temperature conditions in real time during VSD+ treatments (Command III Poultry Management Systems, INC, n.d).

CCTV cameras were used to monitor each room and were 4k ultra-HD resolution dome

cameras, and an NVR recording system was set up to record and discourage entry into rooms until all hens were observed to be deceased or the 3-h limit was reached (4 cameras/aviary section; total of 12 cameras/room) (Lorex Corporation, n.d-a; Lorex Corporation, n.d-b). Each section had two cameras mounted on the bird side wall to record each tier of aviary, one camera mounted on the ceiling for an aerial view of floor area, and one on the man side of aviary to record floor underneath system and tier 1. This camera layout allowed for maximum viewing area within aviary system. Unconsciousness and death were determined by video observation of hens for signs of loss of posture, neuromuscular spasms, and cessation of movement (Webster and Fletcher, 2001; Eramus et al., 2010).

Since a large quantity of video footage was gathered, four observers were trained by an experienced researcher to identify when hens started recumbency (either lateral or sternal) and when all hens were recumbent (interobserver reliability ≥85% between observers). Then, based on analysis from observers, observed times to first and 100% mortality were confirmed by an experienced researcher. To determine the time to first and 100% mortality, an experienced researcher analyzed videos for first and last hen to be seen exhibiting behavioral indicators of death previously described (i.e., loss of posture, neuromuscular spasms, and cessation of movement) (Webster and Fletcher, 2001; Eramus et al., 2010).

***Heaters and boiler***

A salamander-style forced air heater with a heating capacity of 400,000 BTU was utilized as the main source of heat for this experiment (L.B. White, n.d-a). The door to each room was sealed via fire-retardant plywood sheath, in VSD-H and VSD-HS treatments, a hole was made in shape of salamander style forced air heater that was located in the hallway outside of each room during the study. Unfaced fiberglass insulation was placed in empty space between plywood sheath and heater to minimize leaks (Menards, n.d-c). A wall mounted forced air heater within MSU research facility was used as a secondary heat source, primarily to maintain required temperatures during VSD+ treatments, with minimum heating capacity of 50,000 BTU and maximum heating capacity of 100,000 BTU (L.B. White, n.d-b).

A 30 hp low pressure steam boiler was rented to supply steam for the 3-h limit. A 5.08 cm hole the size of the steam pipe was made on one of the wood panels covering air inlet to administer the steam into room. The boiler was located outside of the entry doors of facility

sealed rooms due to operating safety regulations. The boiler operated at 5 PSI during VSD+; as steam was not released under pressure, the pipe was open to the room without the need of diffuser or other equipment. A total of 113.4 kg of saturated steam was delivered per hour (1.9 kg/minute).

### *Statistical analysis*

Data were analyzed using GLM procedure of SPSS 28 (IBM, n.d). Statistical significance was considered at $P<0.05$. The study was a completely randomized design, and the experimental unit was an aviary section (N= 3 aviary sections/room). Data were analyzed for differences in time to first mortality and 100% mortality, mortality location, temperature, and relative humidity between treatments. Means were separated post hoc with Tukey's Least Significant Difference Test.

## RESULTS

### *Observed times for first and 100% mortality*

Mean length of time to achieve first and 100% mortality for all treatments are reported in **Table 3.1**. Observed time to first mortality was shorter ($P<0.0001$) in all steam treatments compared to VSD-H treatment. VSD-S, VSD-HS, and VSD-SH were comparable between one another but quicker ($P<0.0001$) in achieving 100% mortality than VSD-H. For this project, 100% mortality was unachievable for VSD-H within allotted 3-h limit (70% of hens survived).

| Treatment[1] | First Mortality | 100% Mortality |
|---|---|---|
| VSD-H | $^{B}82.7 \pm 5.8$ | $^{B}180.0 \pm 0$ |
| VSD-S | $^{A}56.7 \pm 0.7$ | $^{A}112.3 \pm 13.4$ |
| VSD-HS | $^{A}49.7 \pm 0.3$ | $^{A}83.3 \pm 5.0$ |
| VSD-SH | $^{A}52.0 \pm 1.0$ | $^{A}103.7 \pm 6.17$ |
| ***P*-value[2]** | <0.0001 | <0.0001 |

**Table 3.1.** Mean times (minutes) to observed first and 100% mortality for each VSD+ treatment.
[1]VSD-H= VSD+ heat only; VSD-S= VSD+ steam only; VSD-HS= VSD+ heat (for 30-min) and then steam; VSD-SH= VSD+ steam (for 30-min) and then heat.
[2]Means with different superscripts within column are significantly different $P<0.05$.

***Mortality location within the aviary***

**Table 3.2** provides the location of mortality within aviary system upon completion of each VSD+ treatment. Within the floor area, fewer carcasses were observed in sections of room subjected to VSD-HS (*P*=0.02) compared to VSD-S and VSD-SH, which had an overall higher number of carcasses. In tier 1, VSD-S and VSD-SH had a lower carcass amount, while VSD-HS had higher number of carcasses (*P*=0.006). No differences were detected in tier 2 (*P*=0.248). For nest box level, treatments that utilized steam first (VSD-S and VSD-SH) were comparable in carcass number but lower (*P*=0.024) than VSD-HS. VSD-H results were removed from table 1 due to higher-than-expected survivability which might have skewed results.

| **Treatment**[1] | **Floor** | **Tier 1** | **Tier 2** | **Nest Boxes** |
|---|---|---|---|---|
| VSD-S | $^{A}$111.3 ± 4.3 | $^{B}$18.7 ± 2.0 | 18.0 ± 2.6 | $^{B}$2.0 ± 0 |
| VSD-HS | $^{B}$81.0 ± 5.0 | $^{A}$40.7 ± 0.3 | 17.0 ± 2.1 | $^{A}$11.3 ± 3.2 |
| VSD-SH | $^{A}$115.3 ± 4.7 | $^{B}$22.7 ± 2.9 | 11.7 ± 1.8 | $^{B}$0.3 ± 0.3 |
| ***P*-value**[2] | 0.02 | 0.006 | 0.248 | 0.024 |

**Table 3.2.** Mean number of carcasses in each tier of aviary system after 3-h time limit. [1]VSD-S= VSD+ steam only; VSD-HS= VSD+ heat (for 30-min) and then steam; VSD-SH= VSD+ steam (for 30-min) and then heat.
[2]Means with different superscripts within column are significantly different $P<0.05$.

***Room temperature***

When VSD+ treatments were conducted, average out-of-doors temperature for VSD-H, VSD-S, VSD-HS, and VSD-SH were 22.8°C, 27.2°C, 26.1°C, and 22.8°C, respectively (World Weather Forecast Service, n.d). **Table 3.3** exhibits the room temperature recorded during each VSD+ treatment. The AVMA-specified temperature of 40°C must be achieved within 30-min and all VSD+ treatments were able to reach temperature and no differences (*P*=0.18) were detected between treatments at 30-min.

**Ventilation shutdown with supplemental heat (VSD-H)**

The VSD-H room temperature prior to treatment was 20.1°C and reached a room temperature of 39.3°C within 30-min after the treatment began (**Table 3.3**). A maximum temperature of 48.0°C was obtained at approximately 55-min while during the last half of treatment, the room temperature fluctuated between 44.0°C and 46.0°C. At 85-min, the first mortality was observed at a room temperature of 46.9°C and after 180-min, a room temperature

of 44.8°C was observed for end of treatment, 100% mortality was not obtained within 180-minute time limit for this treatment (**Table 3.1 and Table 3.3**).

**Ventilation shutdown with supplemental steam (VSD-S)**

The addition of steam in VSD-S resulted in a room temperature increase from 23.5°C to 39.3°C after 30-min (**Table 3.3**). Observed time to first mortality was at 55-min at a temperature of 44.8°C, whereas the observed time to one hundred percent mortality was achieved at 110-min at a temperature of 45.7°C (**Table 3.1 and Table 3.3**). Room temperature steadily increased over the duration of VSD-S, reaching a maximum temperature of 47.2°C at approximately 175-min.

**Ventilation shutdown with heat and steam (VSD-HS)**

The room temperature for the times to observed first and 100% mortality were 48.4°C at 50-min and 47.0°C at 85-min (**Table 3.1 and Table 3.3**). The room temperature increase in VSD-HS room was swift and the maximum temperature achieved was 49.5°C after steam was incorporated into the room for approximately 60-min (**Table 3.3**). This trial was stopped after 110-min due to 100% mortality achieved before 3-h limit, hence missing temperature, and relative humidity data for this treatment in **Table 3.3 and Table 3.4**.

**Ventilation shutdown with steam and heat (VSD-SH)**

When steam was utilized first, temperature increased from 22.5°C to 40.5°C within 30-min (**Table 3.3**). After 30-min, the forced air heater (100,000 BTU) was turned on and room temperature rose to 45.5°C by 40-min. Temperature fluctuated between 43.8°C and 45.8°C, with 45.8°C being the maximum temperature attained towards end of treatment at approximately 170-min. Observed first mortality was recorded at a temperature of 43.8°C after 50-min, while 100% mortality was observed at 45.3°C after 105-min (**Table 3.1 and Table 3.3**).

| Time (min) | VSD-H[1] | VSD-S | VSD-HS | VSD-SH | *P*-value[2] |
|---|---|---|---|---|---|
| 0 | $^{A}$20.1 ± 0.1 | $^{C}$23.5 ± 0 | $^{B}$22.3 ± 0.3 | $^{B}$22.5 ± 0 | <0.001 |
| 5 | $^{A}$21.5 ± 0.3 | $^{C}$23.5 ± 0 | $^{BC}$22.6 ± 0.1 | $^{B}$22.5 ± 0 | <0.001 |
| 10 | $^{A}$23.2 ± 0.2 | $^{A}$23.7 ± 0.2 | $^{A}$22.8 ± 0.1 | $^{A}$22.8 ± 0.3 | 0.042 |
| 15 | $^{A}$22.6 ± 0.1 | $^{B}$26.7 ± 0.9 | $^{B}$27.0 ± 1.1 | $^{B}$27.3 ± 1.3 | 0.009 |
| 20 | $^{A}$24.4 ± 0.4 | $^{B}$32.8 ± 1.2 | $^{B}$34.3 ± 2.0 | $^{B}$33.0 ± 1.5 | 0.002 |
| 25 | $^{A}$32.4 ± 1.0 | $^{A}$36.0 ± 1.0 | $^{A}$40.0 ± 2.5 | $^{A}$37.3 ± 1.3 | 0.06 |
| 30 | 39.3 ± 1.5 | 39.3 ± 0.9 | 44.9 ± 2.7 | 40.5 ± 1.0 | 0.181 |

**Table 3.3.** Mean room temperatures (°C) during VSD+ treatments. Environmental parameters were measured every 30-s and means are based on average of 4 data loggers placed throughout each room. [1]VSD-S= VSD+ steam only; VSD-HS= VSD+ heat (for 30-min) and then steam; VSD-SH= VSD+ steam (for 30-min) and then heat.

**Table 3.3 (cont'd)**

| | | | | | |
|---|---|---|---|---|---|
| 35 | 44.8 ± 1.9 | 41.7 ± 0.7 | 47.1 ± 2.4 | 43.0 ± 1.0 | 0.301 |
| 40 | 45.8 ± 2.1 | 43.0 ± 0.8 | 46.3 ± 1.6 | 45.5 ± 1.0 | 0.586 |
| 45 | 43.8 ± 1.8 | 43.7 ± 0.7 | 46.8 ± 1.0 | 45.0 ± 0.5 | 0.325 |
| 50 | 47.9 ± 1.8 | 44.2 ± 0.4 | 48.4 ± 0.9* | 43.8 ± 0.3* | 0.076 |
| 55 | $^{AB}$48.0 ± 1.8 | $^{AB}$44.8 ± 0.3 | $^{B}$49.3 ± 0.8 | $^{A}$43.3 ± 0.3 | 0.041 |
| 60 | $^{AB}$47.1 ± 1.6 | $^{AB}$44.8 ± 0.3* | $^{B}$49.5 ± 0.6 | $^{A}$43.3 ± 0.3 | 0.018 |
| 65 | $^{AB}$46.8 ± 1.5 | $^{A}$44.3 ± 0.3 | $^{B}$49.1 ± 0.4 | $^{A}$43.3 ± 0.3 | 0.012 |
| 70 | $^{AB}$46.6 ± 1.4 | $^{AB}$44.0 ± 0.3 | $^{B}$48.1 ± 0.4 | $^{A}$43.5 ± 0.5 | 0.025 |
| 75 | $^{AB}$46.9 ± 1.2 | $^{A}$43.7 ± 0.2 | $^{B}$47.6 ± 0.4 | $^{AB}$44.0 ± 0.5 | 0.021 |
| 80 | $^{A}$46.9 ± 1.2 | $^{A}$43.5 ± 0.3 | $^{A}$47.0 ± 0.4 | $^{A}$44.3 ± 0.8 | 0.039 |
| 85 | $^{B}$47.1 ± 1.1* | $^{A}$43.2 ± 0.2 | $^{AB}$46.6 ± 0.4** | $^{AB}$44.5 ± 0.5 | 0.016 |
| 90 | $^{B}$47.4 ± 1.2 | $^{A}$43.2 ± 0.2 | $^{AB}$46.1 ± 0.4 | $^{AB}$44.8 ± 0.8 | 0.033 |
| 95 | $^{B}$47.6 ± 1.1 | $^{A}$43.2 ± 0.2 | $^{AB}$45.9 ± 0.2 | $^{AB}$45.3 ± 0.8 | 0.014 |
| 100 | $^{B}$47.8± 1.2 | $^{A}$43.7 ± 0.2 | $^{AB}$45.1 ± 0.4 | $^{AB}$45.5 ± 1.0 | 0.033 |
| 105 | $^{B}$46.4 ± 1.1 | $^{AB}$44.2 ± 0.2 | $^{A}$42.6 ± 0.5 | $^{AB}$45.3 ± 0.8** | 0.03 |
| 110 | $^{B}$45.0 ± 0.9 | $^{B}$45.2 ± 0.2 | $^{A}$39.4 ± 0.7 | $^{B}$45.0 ± 0.5 | <0.001 |
| 115 | 45.0 ± 0.7 | 45.7 ± 0.2** | ----- | 44.8 ± 0.3 | 0.612 |
| 120 | 46.0 ± 0.8 | 46.2 ± 0.2 | ----- | 44.8 ± 0.3 | 0.402 |
| 125 | 46.6 ± 0.9 | 46.5 ± 0.3 | ----- | 44.8 ± 0.3 | 0.295 |
| 130 | 46.6 ± 0.9 | 46.8 ± 0.3 | ----- | 44.8 ± 0.3 | 0.254 |
| 135 | 45.6 ± 0.8 | 46.8 ± 0.3 | ----- | 44.8 ± 0.3 | 0.237 |
| 140 | 44.6 ± 0.8 | 46.7 ± 0.2 | ----- | 45.3 ± 0.3 | 0.144 |
| 145 | 44.0 ± 0.7 | 46.7 ± 0.2 | ----- | 45.3 ± 0.3 | 0.046 |
| 150 | 44.8 ± 0.6 | 46.7 ± 0.2 | ----- | 45.3 ± 0.3 | 0.07 |
| 155 | 45.6 ± 0.7 | 46.7 ± 0.2 | ----- | 45.3 ± 0.3 | 0.279 |
| 160 | 46.4 ± 0.8 | 46.8 ± 0.3 | ----- | 45.3 ± 0.3 | 0.376 |
| 165 | 47.3 ± 0.8 | 46.8 ± 0.3 | ----- | 45.3 ± 0.3 | 0.211 |
| 170 | 46.9 ± 0.9 | 47.0 ± 0.3 | ----- | 45.8 ± 0.3 | 0.547 |
| 175 | 45.8 ± 0.8 | 47.2 ± 0.2 | ----- | 45.8 ± 0.3 | 0.258 |
| 180 | 44.8 ± 0.8 | 47.2 ± 0.2 | ----- | 45.8 ± 0.3 | 0.07 |

**Table 3.3.** Mean room temperatures (°C) during VSD+ treatments. Environmental parameters were measured every 30-s and means are based on average of 4 data loggers placed throughout each room.
[1]VSD-S= VSD+ steam only; VSD-HS= VSD+ heat (for 30-min) and then steam; VSD-SH= VSD+ steam (for 30-min) and then heat.
[2]Means with different superscripts within column are significantly different $P<0.05$.
*Indicates at what time and temperature the first observed mortality occurred for each VSD+ treatment.
**Indicates at what time and temperature 100% observed mortality occurred for each VSD+ treatment (VSD-H never reached 100% mortality).

### *Relative humidity*

Commencing each treatment, out-of-doors average relative humidity for VSD-H, VSD-S, VSD-HS, and VSD-SH were 43.0%, 45.0%, 54.0%, and 49.0%, respectively, all below the 70% RH threshold (World Weather Forecast Service, n.d). **Table 3.4** indicates the relative humidity recorded during each VSD+ treatment. When analyzing relative humidity attained for VSD-H, VSD-S, VSD-HS, and VSD-SH there was a difference ($P<0.001$) between treatments at each time point, with the exception of 5-min (**Table 3.4**). By 40-min, VSD-H had a lower relative

humidity than all other treatments, and there was no difference between the treatments with steam ($P$<0.0001).

**Ventilation shutdown with supplemental heat**

For VSD-H, first mortality was observed at a relative humidity of 38.1% by 85-min, while for 100% mortality was observed at 48.9% after 180-min (**Table 3.1 and Table 3.4**). At 5-min into treatment procedure, relative humidity quickly increased from 59.3% to 70.4%, with 70.4% being the highest obtained for VSD-H (**Table 3.4**). However, as it progressively got hotter in the room, relative humidity decreased to 39.9% after 30-min. After 70-min, relative humidity started to increase and reached 50.1% at approximately 145-min.

**Ventilation shutdown with supplemental steam**

Initial relative humidity recorded for VSD-S was 78.7% and rose to 98.0% after 30-min of steam inclusion (**Table 3.4**). Throughout treatment progression, relative humidity kept steadily increasing until 100% was achieved after 100-min. After 100% was achieved, relative humidity remained constant at 100% for remainder of procedure. When first mortality was observed at minute 55, the relative humidity was 98.7% and for 100% mortality it was 100% after 110-min (**Table 3.1 and Table 3.4**).

**Ventilation shutdown with heat and steam**

Within 30-min of heat introduction in the VSD-HS room, relative humidity quickly decreased from 70.6% to 39.9% (**Table 3.4**). Then after 30-min, the heater was turned off and steam was administered into room. With 15-min of steam inclusion, relative humidity increased to 95.5%. After 55-min, a maximum relative humidity of 97.1% was achieved before slowly decreasing over duration of the treatment, ending at 70.0% in 110-min. First and 100% mortality were observed at a corresponding relative humidity of 97.0% at 50-min and 94.5% at 85-min (**Table 3.1 and Table 3.4**). Since 100% mortality was observed prior to the end of the allotted 3-h time, the treatment was ended.

**Ventilation shutdown with steam and heat**

At 50-min into VSDS-SH, first mortality was observed at a relative humidity of 98.8%, while it took an extra 54-min for 100% mortality to be documented at a relative humidity of 95.8% (**Table 3.1 and Table 3.4**). Since steam was utilized first, relative humidity quickly increased from 67.8% to 97.0% within 30-min (**Table 3.4**). After 30-min, steam pressure was decreased and forced air heater was turned on; relative humidity was elevated to 98.8% within

20-min of heater being on. In latter half of the treatment, relative humidity alternated between 95% and 98%, before reaching a maximum of 99.3% after 160-min.

| Time (min) | VSD-H[1] | VSD-S | VSD-HS | VSD-SH | *P*-value[2] |
|---|---|---|---|---|---|
| 0 | $^{A}$59.3 ± 0.3 | $^{C}$78.7 ± 0.8 | $^{B}$70.6 ± 0.7 | $^{B}$67.8 ± 0.8 | <0.001 |
| 5 | 70.4 ± 6.3 | 80.5 ± 1.0 | 71.4 ± 1.3 | 68.0 ± 1.0 | 0.278 |
| 10 | $^{A}$58.3 ± 0.7 | $^{C}$81.7 ± 0.7 | $^{B}$73.4 ± 1.3 | $^{C}$81.8 ± 5.3 | <0.001 |
| 15 | $^{A}$59.9 ± 0.4 | $^{C}$99.2 ± 0.4 | $^{B}$71.1 ± 3.3 | $^{C}$98.3 ± 0.3 | <0.001 |
| 20 | $^{A}$64.5 ± 1.1 | $^{B}$98.7 ± 0.7 | $^{A}$57.3 ± 5.2 | $^{B}$97.8 ± 0.3 | <0.001 |
| 25 | $^{A}$50.4 ± 2.7 | $^{B}$98.3 ± 0.8 | $^{A}$46.9 ± 5.0 | $^{B}$97.0 ± 0.5 | <0.001 |
| 30 | $^{A}$39.9 ± 2.6 | $^{B}$98.0 ± 0.8 | $^{A}$39.9 ± 4.4 | $^{B}$97.0 ± 0.5 | <0.001 |
| 35 | $^{A}$33.8 ± 2.5 | $^{C}$97.8 ± 0.7 | $^{B}$67.4 ± 8.1 | $^{C}$96.8 ± 0.3 | <0.001 |
| 40 | $^{A}$32.5 ± 2.6 | $^{B}$98.2 ± 0.7 | $^{B}$88.6 ± 5.1 | $^{B}$97.0 ± 0.5 | <0.001 |
| 45 | $^{A}$36.0 ± 2.9 | $^{B}$98.3 ± 0.7 | $^{B}$95.5 ± 2.3 | $^{B}$97.0 ± 0.5 | <0.001 |
| 50 | $^{A}$31.6 ± 2.6 | $^{B}$98.7 ± 0.6 | $^{B}$97.0 ± 1.3* | $^{B}$98.8 ± 0.8* | <0.001 |
| 55 | $^{A}$32.5 ± 2.6 | $^{B}$99.2 ± 0.8 | $^{B}$97.1 ± 0.8 | $^{B}$97.5 ± 0.5 | <0.001 |
| 60 | $^{A}$34.3 ± 2.5 | $^{B}$98.7 ± 0.7* | $^{B}$96.6 ± 0.9 | $^{B}$98.0 ± 0 | <0.001 |
| 65 | $^{A}$35.9 ± 2.4 | $^{B}$99.2 ± 0.6 | $^{B}$95.1 ± 1.4 | $^{B}$98.0 ± 0 | <0.001 |
| 70 | $^{A}$37.0 ± 2.3 | $^{B}$99.5 ± 0.5 | $^{B}$94.5 ± 1.2 | $^{B}$97.8 ± 0.3 | <0.001 |
| 75 | $^{A}$37.8 ± 2.4 | $^{B}$99.5 ± 0.3 | $^{B}$94.4 ± 1.4 | $^{B}$97.8 ± 0.8 | <0.001 |
| 80 | $^{A}$38.1 ± 2.2 | $^{B}$99.5 ± 0.3 | $^{B}$94.5 ± 1.4 | $^{B}$97.3 ± 1.3 | <0.001 |
| 85 | $^{A}$38.3 ± 2.2* | $^{B}$99.5 ± 0.3 | $^{B}$94.9 ± 1.4** | $^{B}$95.8 ± 2.8 | <0.001 |
| 90 | $^{A}$38.1 ± 2.1 | $^{B}$99.8 ± 0.2 | $^{3.4B}$94.9 ± 1.4 | $^{B}$95.3 ± 3.3 | <0.001 |
| 95 | $^{A}$38.3 ± 2.2 | $^{B}$99.8 ± 0.2 | $^{B}$94.8 ± 1.2 | $^{B}$94.5 ± 4.0 | <0.001 |
| 100 | $^{A}$39.1 ± 2.4 | $^{C}$100 ± 0 | $^{B}$82.9 ± 1.9 | $^{C}$95.5 ± 3.0 | <0.001 |
| 105 | $^{A}$42.4 ± 2.4 | $^{C}$100 ± 0 | $^{B}$71.0 ± 0.7 | $^{C}$95.8 ± 2.8** | <0.001 |
| 110 | $^{A}$45.5 ± 2.5 | $^{C}$100 ± 0 | $^{B}$70.0 ± 0.4 | $^{C}$96.5 ± 2.0 | <0.001 |
| 115 | $^{A}$45.0 ± 2.0 | $^{B}$100 ± 0** | ----- | $^{B}$97.3 ± 1.3 | <0.001 |
| 120 | $^{A}$43.4 ± 1.9 | $^{B}$100 ± 0 | ----- | $^{B}$98.0 ± 1.0 | <0.001 |
| 125 | $^{A}$42.9 ± 2.0 | $^{B}$100 ± 0 | ----- | $^{B}$98.3 ± 0.8 | <0.001 |
| 130 | $^{A}$43.9 ± 2.2 | $^{B}$100 ± 0 | ----- | $^{B}$98.5 ± 0.5 | <0.001 |
| 135 | $^{A}$46.6 ± 2.1 | $^{B}$100 ± 0 | ----- | $^{B}$98.5 ± 0.5 | <0.001 |
| 140 | $^{A}$49.3 ± 1.9 | $^{B}$100 ± 0 | ----- | $^{B}$98.8 ± 0.3 | <0.001 |
| 145 | $^{A}$50.1 ± 1.7 | $^{B}$100 ± 0 | ----- | $^{B}$98.8 ± 0.3 | <0.001 |
| 150 | $^{A}$47.5 ± 1.6 | $^{B}$100 ± 0 | ----- | $^{B}$98.8 ± 0.3 | <0.001 |
| 155 | $^{A}$45.1 ± 1.8 | $^{B}$100 ±0 | ----- | $^{B}$99.0 ± 0.5 | <0.001 |
| 160 | $^{A}$43.5 ± 1.9 | $^{B}$100 ± 0 | ----- | $^{B}$99.3 ± 0.3 | <0.001 |
| 165 | $^{A}$41.9 ± 1.9 | $^{B}$100 ± 0 | ----- | $^{B}$99.0 ± 0.5 | <0.001 |
| 170 | $^{A}$43.3 ± 2.0 | $^{B}$100 ± 0 | ----- | $^{B}$99.3 ± 0.3 | <0.001 |
| 175 | $^{A}$46.3 ± 2.1 | $^{B}$100 ± 0 | ----- | $^{B}$99.3 ± 0.3 | <0.001 |
| 180 | $^{A}$48.9 ± 2.2 | $^{B}$100 ± 0 | ----- | $^{B}$99.3 ± 0.3 | <0.001 |

**Table 3.4.** Mean relative humidity (%) during VSD+ treatments.
RH was measured every 30 s and means are based on average of 4 data loggers located throughout each room. [1]VSD-S= VSD+ steam only; VSD-HS= VSD+ heat (for 30-min) and then steam; VSD-SH= VSD+ steam (for 30-min) and then heat.
[2]Means with different superscripts within column are significantly different $P<0.05$.
*Indicates at what time and relative humidity the first observed mortality occurred for each VSD+ treatment.
**Indicates at what time and relative humidity 100% observed mortality occurred for each VSD+ treatment (VSD-H never reached 100% mortality).

## DISCUSSION

In this study, steam was evaluated as a "plus" in ventilation shutdown for the depopulation of laying hens. Based on results, the hypothesis previously stated can be accepted since steam treatments were faster than VSD with heat alone in achieving 100% mortality. During times of high heat and low relative humidity animals can release heat quicker; while with high relative humidity, animal bodies cannot cool as efficiently. This statement is especially true in poultry that use evaporative cooling for heat removal. Authors Saeed et al., (2019) noted that when temperature reached 35°C and relative humidity was 40.0%, birds could remove 80.0% of total body heat through evaporative cooling, whereas at 35.0°C and 50.0% relative humidity, heat loss was reduced by 50%. However, when temperature remained at 35.0°C and relative humidity increased to 100%, birds could no longer remove body heat, causing chronic stress, shock, and high mortality (Saeed et al., 2019).

When poultry are exposed to high ambient temperatures and/or high relative humidity, birds will adjust their behavior and physiological needs to combat heat stress (Daghir, 2008). Qureshi (2001) observed laying hens' reactions to different ambient temperatures and relative humidity. According to the author, hens were undisturbed between 20.0°C to 25.0°C when relative humidity was 75.0%. However, when temperatures reached between 30.0°C and 35.0°C and relative humidity increased to 100%, hens were moderately disturbed; while in temperatures beyond 40.0°C with a relatively humidity of 100%, hens were extremely disturbed, and death occurred (Qureshi, 2001). Results from present study concurred with observations from Qureshi (2001), since recorded temperatures for all treatments were above 40°C; although, steam treatments were the only ones capable of reaching a relative humidity in the 90% to 100% range, resulting in 100% mortality. Treatment VSD-H was unable to reach 100% mortality in 3-h, which may be due to treatment unable to reach a high enough relative humidity throughout procedure, probably because hens were able to still dissipate heat during observed high temperatures. Lee et al., (1945), noted several relationships between temperature and relative humidity that should be worthy of consideration. When relative humidity is kept at 75% or less, hens can tolerate a temperature of 37.8°C or above for a 7-h period (Lee et al., 1945). Additionally, if temperature increases to 40.6°C, hens can tolerate this temperature for only a few hours regardless of the relative humidity, however relative humidity becomes an essential factor for hen survival in temperatures of 40.6°C and above (Lee et al., 1945).

Moreover, it is essential to recognize that by the time first mortality was observed for VSD-H, all VSD+ treatments that utilized steam had already achieved their first observed mortality approximately 30-min faster. VSD-HS was the quickest to achieve 100% observed mortality; this was also approximately at the same time that the first observed mortality was recorded for VSD-H. VSD-HS and VSD-SH first mortality was observed at approximately the same time, within 2-min of each other. These results indicate that ventilation shutdown with steam inclusion was a more effective way to depopulate laying hens than VSD with supplemental heat only. When supplemental heat was utilized first, coupled with steam addition after 30-min, a quicker time to mortality was achieved (100% observed mortality in less than 90 minutes).

Furthermore, all VSD+ treatments were able to achieve the AVMA-required temperature of 40.0°C and remained comparable between one another throughout the 3-h limit, with little variation in temperature. However, when comparing the relative humidity table between treatments, variability was apparent. In VSD-S, when only steam was utilized, relative humidity increased rapidly, close to 100%, and maintained stable throughout duration of procedure and VSD-SH followed a similar trend. For VSD-HS, a distinction can be made of when steam was administered into room, when relative humidity increased from 39.9% to 67.4% after 5-min of steam addition, while relative humidity decreased over time when only supplemental heat was used (VSD-H). A simultaneous rise in both room temperature and relative humidity likely contributed to hen's inability to properly remove body heat through evaporative cooling.

When mortality location within the aviary system was evaluated after depopulation, VSD-S and VSD-SH had an overall greater number of carcasses in the floor area compared to other areas of the system. Based on these results, carcass removal after VSD+ with steam application may be less challenging for workers than other depopulation methods since the majority of carcasses would be located in floor area, and outside of the system. This might be particularly true if $CO_2$ was utilized, as chickens can detect low concentrations of $CO_2$, thus hens might move into the multi-tier aviary and out of floor area and eventually die inside aviary (Raj and Gregory, 1991; Sandilands et al., 2011).

In the current study, VSD+ treatments that utilized steam were able to achieve both high ambient temperature and relative humidity, thus leading to a much faster time to death than when only high ambient temperature was achieved in VSD-H. These findings agree with previous

research that having a high relative humidity, along with elevated temperatures, are both crucial in attaining 100% mortality during ventilation shutdown (Zhao et al., 2019). Additionally, results from present study concur with Baysinger et al., (2021), showing addition of steam to be effective and essential in success of ventilation shutdown. Together, these findings could potentially guide use of this method for mass depopulation in event of a foreign animal disease outbreak or severe market disruption (Baysinger et al., 2021). Moreover, the current findings for time to observed 100% mortality are comparable to published results obtained from ventilation shutdown with carbon dioxide (Eberle-Krish et al., 2018). Steam usage has advantages compared to $CO_2$, such as worker safety, possibly lower cost, and there are minimal concerns for steam shortage as experienced with $CO_2$ shortages (Eberle-Krish et al., 2018; Burgess, 2022; CGA, n.d; HSE, n.d).

## CONCLUSION

In conclusion, steam alone or in combination with heat, demonstrated to be effective as a "plus" in ventilation shutdown for depopulation of laying hens in a cage-free system. Since steam was capable of causing a simultaneous rise in relative humidity and ambient temperature; steam usage could potentially eliminate environmental differences to allow for a more uniform mortality spread and perhaps make VSD+ more consistent. When compared with the current "gold" standard of VSD+ heat and $CO_2$, addition of steam to a VSD+ procedure would be beneficial since VSD+ treatments that utilized steam had comparable mortality times to VSD with $CO_2$. The observed high relative humidity combined with high temperature in VSD+ steam treatments allowed for an expediated depopulation response. Having another alternative to employ during mass depopulation with VSD+ would aid in ensuring HPAI virus is "stamped out" during critical depopulation period (24-h to 48-h) USDA has outlined. While data from present study gives an insight to how successful steam addition is during VSD+ depopulation, more research must be conducted in a commercial environment.

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# CHAPTER 4

# IMPLICATIONS AND CONCLUSIONS

## IMPLICATIONS AND CONCLUSIONS

This research was to explore different management strategies to make mass depopulation more efficient in cage-free housing systems. Experiment one utilized a novel approach to induce a movement response from hens by usage of UV light flash alone or combined with darkening of floor area, to gather hens in floor area and out of an aviary system. While the data presented from this experiment demonstrates that UV light flash can be used to move hens to desired location (i.e., floor area), this may not be effective for long-term movement during mass depopulation. One way that might be beneficial of applying UV light flash is by using it, so hens move to desired location, and then closing either aviary (if doors are present) or fencing off area in barn so hens do not retrieve to original location. Another interesting finding was that although hens in UV treatments exhibited more stress related behaviors (standing alert), hens were still comfortable in the environment, indicating that UV light flash may be used as a non-stress inducing tool for short-term movement. Moreover, UV light may be utilized to prevent undesired behaviors (i.e., piling and floor eggs) since UV lights comes in several forms (light bars, string lights, bulbs, etc.). However, more research must be conducted to learn if there are any long-term effects of technique and if it can be applicable to prevent undesired behaviors.

Experiment two, explored effectiveness of steam addition, during a ventilation shutdown with heat (VSD+) procedure, as an alternative "plus" of depopulation in the event of a foreign animal disease outbreak (FAD). The data presented from this experiment provides a first look at utilization of steam during VSD+ for mass depopulation of laying hens in a cage-free system. Investigating alternative "pluses" to VSD+ is crucial because, realistically, the feasible depopulation methods that can be implemented in a commercial setting are water-based foam, gassing, and VSD+ heat and/or $CO_2$. However, foam method cannot be applied in presence of metal structures (aviaries), thus gassing and VSD+ heat and/or $CO_2$ are the feasible options for table egg producers. Additionally, procurement of $CO_2$ is challenging and costly; so really, VSD+ heat and/or $CO_2$ is the only viable option for egg producers. Utilization of VSD+ for depopulation has only been approved since 2015 and use is reserved as a last resort method, little research has been conducted on either experimental or field scale, so refinement is needed. Results revealed steam to be a suitable plus for VSD+, as it resulted in significantly shorter depopulation times compared to ventilation shutdown with supplemental heat. Moreover, steam acquisition (if to be used during a FAD outbreak) may not be as challenging, since steam is

utilized in the food and beverage industry, thus equipment is available for emergency situations. As VSD+ keeps being implemented in response to FAD outbreaks, USDA-APHIS and AVMA need evidence-based alternative depopulation methods that will permit poultry producers to have options while taking into account the welfare of infected birds.