STRESS AND EFFICACY OF GASEOUS EUTHANASIA UNDER VARIOUS LED LIGHTING FOR NEONATAL CHICKS AND DUCKLINGS AND COMPARING ON-FARM EUTHANASIA METHODS FOR DUCKS AND TURKEYS

A Thesis

by

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ABSTRACT

Euthanasia in poultry is a major welfare concern and optimizing the methods on-farm, at the hatchery and at the processing plant is paramount. Lighting greatly affects poultry behavior and welfare therefore the lighting conditions during euthanasia may be one method to improve welfare. Furthermore, on farm euthanasia can often be difficult so finding the most effective method which is also the most user friendly is also important for optimal welfare. The first experiment aimed to investigate the use of different lighting conditions during the use of CO_2 and N_2 during neonatal poultry euthanasia. The second experiment investigated the use of different colors of light during stunning of broiler chickens and their effect on stress hormones. The third experiment consisted of on-farm euthanasia of turkeys both 8 weeks and 12 weeks of age using various captive bolt devices and cervical dislocation methods. The final experiment compared the efficacy of an experimental crossbow with a known effective captive bolt Zephyr-EXL on market age Pekin ducks. The first experiment showed carbon dioxide combined with the absence of light was the optimum euthanasia condition for neonates. Experiment two, demonstrated that green lighting had the lowest CORT concentration for both broiler chickens and Pekin ducks and is considered effective in reducing stress on poultry prior to slaughter. Experiment 3 and 4 both demonstrated that while all euthanasia methods resulted in successful euthanasia, the captive bolt devices were more effective and easier to perform proper euthanasia. Furthermore, the experimental crossbow was indicated to be the best method overall. In conclusion, CO_2 in conjunction with the absence of light is considered the most effective and humane method of neonatal euthanasia, while green light should be implemented during pre-slaughter. On-farm euthanasia showed captive bolt devices are more effective and humane than cervical dislocation methods. The Experimental Crossbow was similar to other captive bolts and should also be considered as an effective euthanasia device.

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NOMENCLATURE

ACTH	Adrenocorticotropic hormone
BRM	Broomstick
CORT	Corticosterone
COM	Cessation of Movement
CP	Cutaneous Penetration
CRS	Experimental Crossbow
JAR	Jarvis Pneumatic Stunner
KED	Mechanical Cervical Dislocation Device
LOP	Loss of Posture
MAN	Manual Cervical Dislocation
NMR	Nictitating Membrane Response
PLR	Pupillary Light Reflex
P/M	Difference in Loss of Posture to Cessation of Movement
TED	Turkey Euthanasia Device
VOC	Vocalizations
V-Time	Vocalizations/Cessation of Movement
ZEP	Zephyr-EXL

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CHAPTER I

INTRODUCTION

Routine on-farm euthanasia is a necessity for the prevention of potential disease outbreak and continuous suffering of injured or sick birds (Sparrey et al., 2014). Culling methods should minimize pain and distress, followed by rapid insensibility and death via loss of respiratory function and cardiac arrest (Woolcott et al., 2018). An effective euthanasia method causes death by 3 basic mechanisms: direct depression of the neurons necessary for life functions, hypoxia and/or the physical disruption of the brain activity (AVMA, 2020).

There are not any federal regulations regarding the rearing, transportation, breeding and slaughter of poultry in the United States from a welfare perspective. Animal welfare reform however began in the United States with the Animal Welfare Act, (U.S.S. 2131-2159) which set standard for the humane care and handling of certain animal species excluding poultry. The Humane Slaughter Act, (U.S.S. 1901-1906) requires that all livestock be slaughtered in a humane method to prevent continuous suffering but again does not cover poultry. Other types of welfare based federal legislation are is the Twenty-Eight-Hour-Law (U.S.S 80502) which prohibits the transportation of livestock across state lines for more than twenty-eight hours without being unloaded for at least five hours of rest. The majority of welfare laws on poultry are passed through the state legislation. All three federal legislative acts do not include poultry and other birds. State legislation does have regulations on the welfare of poultry; these protections are severely limited and vary state to state. Additionally, regulations are based around the slaughter of livestock animals to ensure hygienic preparation of the meat. When referring to the euthanasia of neonatal chicks, they are not consumed and as a result, lack any public health attention (Jaksch, 1981).

Within the United States, all fifty states have a form of anti-cruelty laws, mostly for the protection of inhumane treatment and suffering of livestock including poultry. Thirty of the states exclude farm animals (poultry). The California Humane Slaughter Act specifies that all poultry must me rendered insensible to pain before killing. These Killing methods listed by this law include gassing with carbon dioxide, electrical stunning, electrocution, cervical dislocation and decapitation.

The American Veterinary Medicine Association (AVMA) contains guidelines for proper welfare management of poultry in commercial settings. Animals must be provided water, food, proper handling and health care. The AVMA has set principals that specifically detail the slaughter procedures for poultry. In order to have proper euthanasia of poultry they must comply with the two statements "their humane disposition to induce death in a manner that is in accord with an animals interest and/or because it is a matter of welfare, and the use of humane techniques to induce the most rapid and painless distress-free death possible" (AVMA, 2020). Federal legislation put forth these laws to protect the basic welfare of farmed animals. Feeding, watering, environmental protection and killing of animals are outlined within these guidelines. Livestock animals however have been determined to be an exception to these laws, specifically poultry.

Modern poultry has developed two specific hybrid breeds of chickens, meat broiler chickens are specialized for maximum growth efficiency, and laying flocks are designed for maximum egg productivity (Jaksch, 1981). Alongside to the increase in genetic diversity, male layer chicks are not feasible economically and as a result, the newly hatched chicks are euthanized in the hatchery (Gerken et al., 2003). The AVMA (2020) lists inhaled agents include such as carbon dioxide, nitrogen gas, argon and carbon monoxide, as well as maceration as acceptable means of euthanasia. Despite the use of gaseous euthanasia as an approved method, most hatcheries within the United States use maceration. Maceration is the use of a specially designed machine which contain rotating blades that will cause immediate fragmentation and death. Macerations is believed to be equivalent to cervical dislocation and cranial compression which are the preferred method of euthanasia according to the AVMA, Federation of Animal Science Societies, Agriculture Canada, World Organization for Animal Health and the European Council.

In regard to the production of turkeys, each commercial turkey grow-out facility is required to have a protocol to address sick or injured birds. Third party audits can serve as regulations model for poultry producers dealing with sick or injured birds. One such third party auditor, the Global Animal Partnership, has a set list of standards for the raising of turkeys, specifically on-farm euthanasia of turkeys. Global Animal Partnership standard guidelines and The National Turkey Federation in the United States approve cervical dislocation is a method for turkeys up to the age of five weeks. Mechanical cervical dislocation is not approved at any age, while non-penetrating captive bolts and gas killing systems (use of CO_2) are approved throughout the entire grow out period of the birds (GAP, 2015; NTF, 2020).

Most guidelines and protocols follow the published works of the American Veterinary Medical Association, however methods that are considered acceptable by the AVMA may not be practical or suitable for on-farm euthanasia (Erasmus, 2009). Turkey producers may face a more challenging task as turkeys are a larger commercially grown poultry species.

CHAPTER II

LITERATURE REVIEW

Welfare Concerns With Euthanasia

Under commercial conditions, birds are routinely euthanized on-farm due to illness or injury (Martin et. Al., 2018). The most important criteria for any euthanasia program is if the animal experiences pain during the method. When assessing pain in animals, the use of an analgesic can aide in reducing a particular procedure that may cause distress and pain, however it is impossible to decipher pain in animals. Unlike analgesics methods, birds do not recover from euthanasia (Erasmus, 2009). Animals that are being euthanized may already be experiencing pain and distress, thus adding to the complications of assessing welfare implications of the method. If it can be determined that any particular method causes immediate or swift insensibility, the need to assess pain and distress during or from pre-existing methods will be unnecessary (Erasmus, 2009).

Two types of stress exist, eustress which is the normal or average psychological stress that is deemed beneficial for the animal, and distress which is the negative stress type and is the psychological combination of pain and fear (Zulkifli and Siegel, 1995; Martin et. al, 2018). Distressing farm animals, particularly in poultry, come from handling, crating and transporting the animals. An ideal welfare-based euthanasia method will allow an animal to be restrained with a little pain and distress all while resulting in rapid and irreversible insensibility and death (Blackmore, 1993; Erasmus, 2009).

Operator wellbeing and safety should be considered when evaluating killing methods. According to the AVMA, criteria for evaluating euthanasia methods other than previously stated should include safety of personnel, documented emotional effect on observers or operators, drug availability and human abuse potential (AVMA, 2020). Personnel who will perform euthanasia must be trained and demonstrate proficiency when applying a technique while being closely observed by a supervisor (AVMA, 2020). Physical euthanasia methods require the operator to have a more direct association between the operator and the animal, which can be considered offensive to and upsetting for the operator (AVMA, 2020). While physical methods of euthanasia require the operator to restrain and properly kill the animal, other methods do not require the direct association, but are considered visually displeasing to the operator. When performing maceration, neonatal birds are physically fragmented via rotating blades. This process may be aesthetically unpleasant for certain operators and observers. It has also been observed that distress may develop among personnel directly involved in repeated killing, which may involve a psychological state including a sense of work dissatisfaction or alienations (AVMA, 2020).

When addressing euthanasia, public consideration and expectations must be applied and play a role when considering euthanasia methods. Public exposure to zoo animals, animals involved in roadside accidents, stranded marine animals and injured wildlife may draw public attention and the approach should be considered when the animals are killed (AVMA, 2020). The public perceptions should be considered; however, the primary responsibility is to do what is in the animals bet interest under the circumstances. The psychological well-being of the human participants and the physical safety of personnel when handling the animals and performing proper euthanasia should be protected (AVMA, 2020).

Neuroanatomy Of The Avian Brain

The avian brain can be divided into three main sections, the telencephalon (cerebrum), the metencephalon (cerebellum), and the brainstem (Erasmus, 2009). The brainstem connects the cranial section of the brain to the spinal cord and is comprised of the medulla oblongata, pons, mesencephalon (midbrain) and diencephalon (Breazile and Hartwig, 1989; Erasmus, 2009). The cerebrum controls motor function by processing sensory information. The cerebrum can be further separated into two sections, the left and the right hemispheres (Jarvis et. al., 2005). These two hemispheres are responsible for processing visual and auditory information (Nickel et. al., 1977).

The cerebellum in the avian brain is similar to the mammalian brain and is necessary for locomotion functions and balance (Erasmus, 2009). The cerebellum is divided into lobes varying in size which is dependent on the species and is comprised of three structural layers, the molecular layer, motor ganglions layer and the granular layer (Karten et. al., 1974).

The midbrain's main function is to organize any stimulation that may come from the environment. The midbrain contains the tegmentum and the optic tectum regions. The tegmentum aids in controlling the locomotion, and the optic tectum aids in processing the visual stimuli (Nickel et. al., 1977).

All stimuli information from the cerebrum and cerebellum are directed in the pons. The pons is positioned just above the medulla oblongata, which controls respiration, circulation and all bodily motor functions including food intake (Nickel et. al., 1977). The medulla is comprised of grey matter and ganglion cells, which is collectively called the reticular formation. Through several neural networks, the reticular formation connects the cerebellum, medulla and the spinal cord (Nickel et. al., 1977). Ascending neural networks extend into the thalamus and the hypothalamus, and descending neural networks connecting to the spinal cord where sensory and motor activity are controlled (Magoun and Rhines, 1946; Erasmus, 2009). The olivary complex is a structure within the reticular formation and helps control locomotion and flight as well as balance (Nickel et. al., 1977). The reticular formation is an integral part of how avian species interpret stimulation and coordinates movement and autonomic function and is responsible for consciousness (Erasmus, 2009).

Determining whether an animal is in a state of unconsciousness or consciousness is extremely important specifically for veterinarians where consciousness must be monitored when performing surgery (Erasmus, 2009).

Lighting Effects On Fear And Stress

Vision is considered the dominant sense of domestic fowl; therefore, special lighting techniques are routinely utilized in the commercial poultry industry (Prescott et. al., 2001). Two types of receptors cells are contained within the retina. Rod cells within the eye are specialized for vision under low light conditions (>0.4 lux) and do not have the ability to distinguish between colors. Cones cells respond to light with intensities greater than 0.4 lux to a maximal intensity of 44 lux and distinguish between colors (Kavtarashvili et. al., 2006; Parvin et. al., 2013). Poultry contain four types of cones in the retina of the eye while humans are trichromatic, indicating that poultry will perceive light differently (Lewis et. al., 2000). The three cones within the human eye with sensitivities to the wavelengths of the primary colors, blue/violet (~450 nm), green (~550 nm) and red (~700 nm). The combination of all three produces white light (Lewis et. al., 2000). Poultry contain a fourth type of cone that contain oil droplets which allows them to perceive part of the ultraviolet range (Bowmaker et. al., 1997; Lewis et. al., 2000). Poultry also have special sensitivities to similar wavelengths of the human spectrum, which may lead to perception of certain colors as brighter than how humans may perceive it (Lewis et. al., 2000). Illuminance from different light sources may also be perceived differently in poultry than humans. A light meter will produce similar readings to humans; however, birds may perceive a greater luminescence with special sensitivities to wavelengths 400-480 nm (blue) and 580-700 nm (red) parts of the spectrum (Nuboer et. al., 1992; Lewis et. al., 2000).

Lighting characteristics such as intensities, spectrum and durations can have major implications on poultry behavior. Welfare is associated with the animal's behavior and should be considered when developing a lighting program (Parvin et. al., 2013). Poultry have been researched using varied colors including red, blue, green and white light. Lighting used in poultry can have profound differences in the behavior and stress on commercial broilers. Published research articles as well as ongoing research have been conducted using lighting along with various methods of euthanasia to generate a greater understanding on stress of the chicken. The use of ideal lighting is an integral part of the overall welfare of the chicken. Aggressiveness and reduced weight gain are associated with high light intensities (~150 lux). Feather pecking, and fighting are all great welfare concerns on poultry due to various lighting techniques (Prayitno et. al., 1997). Special attention should also be considered when referring to birds' photoperiods. Intermittent light schedules have been shown to improve the welfare of turkeys and broiler in contrast to poultry raised under invariable lighting conditions (Parvin et. al., 2013). In addition, chickens exposed to light under 24-hour conditions were associated with elevated stress and disrupted sleep patterns, and turkeys experienced an increase in leg disorders and metabolic diseases. (Classen et. al., 1994; Gordon, 1994; Parvin et. al., 2013). Rearing poultry under certain wavelengths can have adverse effects on behavior of the chicken. Birds reared in red and white light had more activity than any other color. Aggression has been known to coincide with activity (Archer et. al., 2014). Lighting shows to have effects on bird behavior, which could influence stress. A study by Manser (1996) reported that broiler aggression was found to be elevated under red light, in contrast aggression was found to be lowest under blue light. Similar to broilers, turkey aggression was lower under blue light compared to white, red or green light (Manser, 1996). Lighting sources also play a part in the performance and welfare of poultry species. Lewis and Morris (1998) conducted a study comparing lighting types and their effect on leg disorders, where fluorescent lighting showed to have fewer instances of the disorders than birds subjected to incandescent lighting (Lewis and Morris, 1998; Parvin et al., 2013). Light emitting diodes have also been shown to improve welfare of poultry. In a study by Hunt (2009), birds demonstrated calm behaviors where feather pecking, and aggression were reduced under LED lighting. Bird's raised under LED lighting were also recorded to have a decrease instances of mortality (Hunt, 2009). While color differences were not demonstrated, the use of LED lights in conjunction with color types may have an increased effect.

When birds have reached slaughter age, the birds are harvested, loaded onto a transport truck and shackled at the processing plant. When birds are shackled, processing plant workers will grab the birds which may elicit behaviors such as wing flapping, struggling, and vocalizations which are indicators of stress and fear (Jones, 1996). The birds are shackled in a dimly lit room to reduce fear and stress responses. The risk of injury is increased when birds struggle and perform wing flapping, in addition to the added stress carcass quality may decrease resulting in economic losses. (Gregory and Wilkins, 1989). Jones (1996) performed a study comparing whether poultry hooded prior to shackling exhibit similar behaviors associated with stress and fear. Birds exhibited substantially lower instances of vocalizations, wing flapping and signs of struggling when compared to unhooded birds (Jones, 1996). The same concept was further studied on older age broilers, where results were similar to previous study where hooded birds had fewer vocalizations and wing flapping instances (Jones, 1996). Current harvesting conditions require lighting to be dim, under the acknowledgment that low light conditions are considered to have a calming effect where birds are more subdued (Gregory and Bell, 1987). Understanding that birds' cones allow them to have higher sensitivities to red and blue light wavelengths, color type should also be considered.

Euthanasia Of Neonatal Poultry

Undesired hatched chicks are routinely euthanized at the hatchery. One of the main methods to mass depopulate the chicks is the use of special equipment designed to mechanically separate and fragment the brain. The machines are equipped with rotating blades and/or projections and is considered an acceptable form of euthanasia for piped eggs and chicks up to 72 hours of age. Maceration causes immediate death with minimal pain and distress (AVMA, 2020). Maceration is considered to be equivalent to cervical dislocation and cranial compression. Maceration is safe to use for trained personnel and efficient in killing large numbers of poultry. Equipment is required to be kept in perfect working condition, operators are required to be trained to use the maceration equipment and fragmented tissues not properly sanitized can cause a biosecurity risk (Raj et. al., 1995; AVMA, 2020). Males culled are euthanized with specialized equipment via maceration. Maceration causes instantaneous death however, utilizing the method results in tissues, organs and bones to fragment and may not be considered to be visually appealing to the operator (Baker et. al., 2019). The public conception of maceration is forcing alternative methods to be researched.

Alternative Neonatal Euthanasia Methods

Current practices of euthanizing day-old chicks are not a generally well-known topic to the public. One article debated the topic of raising male layers based on carcass characteristics. A simple feeding trial was accomplished comparing commercial broilers and Hy-line brown male layers. The two performance parameters of raising the chickens were average daily gain, average daily feed intake. The average daily gain in commercial broilers maintained the highest amounts of gain with Hy-line brown males' slightly below average (Gerken et. al., 2003). Average daily feed intake also was higher in commercial broilers. Breast percentages of the Hy-line brown male layers were less profitable compared to the commercial broiler; however, the legs and wing percentages were higher than commercial broilers due to overseas market demand of poultry feet, legs and wings. This article contains information that the use of male layer chickens can be used in commercial settings and still be profitable.

The current wave of animal welfare speculation on the industry is pushing poultry producers to change the way layers are handled after hatch. Several methods have been proposed as an alternative to euthanasia. The three main categories that exist include looking into the egg, changing the hen and genetic modification (Leenstra et. al., 2011). Leenstra and coworkers (2011) conducted a focus group discussion to gain insight into opinions of these topics. All groups provided positive feedback with the induction of a dual-purpose chicken, however groups agreed that a dual-purpose chicken was not realistic. Introducing the idea of genetic modification was controversial between groups. Most groups discussed the advantages and disadvantages such as risks to human health and bird welfare impacts. Influencing the hen to produce female offspring only was considered an attractive option, with a few groups against the "manipulation of the hen" (Leenstra et. al., 2011). If poultry producers can produce productive males, an opportunity for millions of viable birds to enter the consumer market can mean the difference in thousands of dollars generated annually. Costs and price were frequently discussed within groups, with the general consensus that people are willing to pay more to prevent the killing of neonatal chicks (Leenstra et. al., 2011).

Gas inhalation

According to the AVMA (2020) carbon dioxide (CO_2), nitrogen (N_2), argon (Ar) and carbon monoxide (CO) are all acceptable methods of gaseous euthanasia. Neonatal birds may be more accustomed to high concentrations of CO_2 due to incubator environments (up to 14%). The concentrations required for quick euthanasia must be in the range of 80% - 90% (Raj et. al., 1995). Carbon dioxide can cause involuntary wing flapping and other terminal movements, which can be unpleasant to the observer. Nitrogen is an odorless, colorless gas also used to euthanize birds. Unlike CO_2, N_2 requires substantially higher concentrations to have proper euthanasia (AVMA, 2020). When preforming gas inhalation, chicks are either exposed to an immersed pre-filled gas chamber or by placing the chick into the gas chamber and gradually filling the chamber until death is determined (Baker et. al., 2019). Carbon dioxide in concentrations higher than 12% will act directly on the central nervous system (Lambooij et. al., 1999). Carbon dioxide is recognized as an anesthetic gas and will induce swift unconsciousness (Lambooij et. al., 1999). An experiment by (Lambooij et. al., 1999) tested carbon dioxide and argon gases ability to effectively euthanize day old chicks. Gasping, wing flapping were observed as fear tests, while loss of posture and eventual cessation of movement were timed to determine the efficacy of gas inhalation. Birds exposed to carbon dioxide at 30% expressed depression of activity and swift onset of unconsciousness. The birds wing flapping was observed prior to the onset of loss of posture suggesting that wing flapping during immersion is a direct response to an anoxic condition. (Lambooij et. al., 1999). While the birds were exposed to argon gas at 60% concentration, loss of motor control became apparent while the chick was still conscious. The observation can be attributed to distress on the bird. Wing flapping and gasping prior to unconsciousness are both means to test the fear in the bird. A direct correlation of these fear tests can be attributed to stress as well. While the day-old chicks are subjected to numerous stressors on their first day after hatching, it is critical to minimize these occurrences. The use of carbon dioxide induces rapid unconsciousness. When birds are introduced to a high concentration of carbon dioxide, it can induce breathlessness and sharp pain in the nasal mucosal membranes. These are indicators of the formation of carbonic acid in the mucous membrane (Lucke, 1979).

Nitrogen gas comprises 78% of normal atmospheric air and can be used for euthanasia by displacing the oxygen (O_2) within the container and blocking the uptake of O_2 by the red blood cells in the lungs (AVMA, 2020). Gerritzen and coworkers (2004) reported that chicken did not avoid chambers with concentration of oxygen less than 2% (Gerritzen et. al., 2004). In addition, when birds were exposed to nitrogen gas less than 98% lost consciousness and showed no signs of distress, with chickens and turkeys showing decreased signs of head shaking and open beak breathing when compared to CO_2 exposed birds (Gerritzen et. al., 2004; AVMA, 2020). When birds were exposed to high concentrations of nitrogen, chickens and turkeys died due to hypoxia. Hypoxia occurs when oxygen delivery is halted to the tissues of the animal, resulting in unconsciousness and subsequent death (AVMA, 2020). When euthanizing birds with nitrogen gas, each chamber must have the nitrogen washed out to prevent exposure of the next bird to a hypoxic condition prior to unconsciousness. The gas displacement rate is also critical to achieve a humane application, by utilizing a regulator and flow meter to generate a consistent gas displacement rate relative to the container (AVMA, 2020).

On-Farm Euthanasia: Classifications

According to (AVMA, 2020), euthanasia agents cause death by three different mechanisms: direct depression of the neurons that are necessary for function of life, hypoxia or a reduced flow of oxygen to the brain, and the physical disruption of total brain activity. In addition, other methods classified as exemptible for euthanasia of poultry species include the use of a penetrating and non-penetrating captive bolt pistol or gunshot, blunt force trauma and cervical dislocation. Inhalant euthanasia agents included the use of gases such as carbon dioxide, carbon monoxide, nitrogen and argon and pharmaceutical agents such as injectable compounds which are not typically used for on farm euthanasia (Erasmus, 2009; AVMA, 2020).

Birds may have to be euthanized to prevent a disease from spreading from bird to bird or remove sick or injured birds from a flock. This spans all types of poultry production from back yard chickens to commercially grown poultry (Martin et. al., 2016). Emergency euthanasia of large animals is mostly performed by using whole-house gas methods or birds are transported to a slaughter facility to be euthanized (Martin et. al., 2016). However, on-farm euthanasia contains two main methods of killing. Cervical dislocation which is designed to cause death by cerebral ischemia and damage to the spinal cord and brain stem (Bader et. al., 2014; Erasmus et. al., 2010a; Martin et. al., 2016). Percussive devices that when used cause extensive brain damage, resulting in loss of brain activity controlling function of vital organs (Martin et. al., 2016; Mason et. al., 2009; Sparrey et. al., 2014). A rapid loss of insensibility is induced when the brain activity is disrupted through a concussive force, or the destruction of the brain tissues (AVMA, 2020). Hypoxia does not result in immediate insensibility, suggesting that physical euthanasia methods may have greater welfare benefits.

The National Turkey Federation (NTF) advocates for cervical dislocation to be the preferred method of euthanasia for turkeys under three kilograms, and non-penetrating captive bolts are preferred method for turkeys over 3 kilograms (NTF, 2013). Additionally, the National Chicken Council (NCC) approves of all methods of euthanasia approved by AVMA (2020) which includes penetrating and non-penetrating captive bolt pistol or gunshot, blunt force trauma and cervical dislocation. Gas inhalant euthanasia agents included are the use of gases such as carbon dioxide, carbon monoxide, nitrogen and argon and pharmaceutical agents such as injectable compounds. However, barbiturates are time consuming, and are controlled substances, and may not be preferred for on-farm practicality (NCC, 2013; AVMA, 2020). While maceration is an approved method of the NCC, gas inhalation is a preferred to euthanize neonatal chicks (>72 hours of age).

Some poultry producers follow euthanasia guidelines set forth by third party audits. The Global Animal Partnership (GAP) allows manual cervical dislocation, penetrating/non-penetrating captive bolts, gas stunning (with the exception of carbon monoxide) and veterinarian administered overdose of injectable anesthetics as acceptable euthanasia methods (GAP, 2017). Mechanical cervical dislocation (equipment used as a cervical dislocation method), applied blunt force trauma, decapitation and de-braining (inserting a sharp object through the roof of the mouth to disrupt brain activity) are not approved euthanasia methods for poultry (GAP, 2017). Other third-party audits such as the Animal Welfare Institute (AWI) and United States Department of Agriculture (USDA) have accepted euthanasia methods approved by the AVMA (2020). Humane Farm Animal Care (HFAC) only approve cervical dislocation and gas inhalation with carbon dioxide or mixture of carbon dioxide and argon gas (HFAC, 2018). Third party audits are not required for poultry producers; however, all producers are required to utilize euthanasia methods approved by the AVMA.

Gas Inhalation

One of the most common uses of gas euthanasia on turkeys is to prevent the outbreak of disease from one barn to another. Due to potentially large size and large numbers of birds, the whole barn can be completely gassed without catching and restraint (Erasmus, 2009). Various gas mixes and single gas atmospheres have been tested (Raj et. al., 2006). Gas inhalation works by creating anoxic conditions, causing hypoxia, respiratory depression and death (AVMA, 2020). Gas mixtures are deemed more favorable compared to high concentrations of inert gasses (Raj et. al., 2006). Argon gas and nitrogen gas are both odorless and may not have a negative welfare correlation when inducted (Raj et. al., 1991). A mixture of gasses such as argon at high concentration and carbon dioxide or nitrogen have proven to induce rapid loss of brain function in turkeys (Raj et. al., 1994). Carbon dioxide causes death by blocking oxygen from entering the blood stream, ultimately causing death by cerebral hypoxia (Raj et. al., 1995). Nitrogen gas is an odorless and tasteless gas that comprises 78% of normal atmospheric air and is used for euthanasia by blocking the uptake of O_2 by the red blood cells in the lungs causing cerebral hypoxia and death (AVMA, 2020). Carbon dioxide has also been associated with involuntary wing flapping and terminal movements, which can be considered unpleasant to the observer (Raj et. al., 1995).

While gas euthanasia can be an effective means of euthanasia, factors such as human safety and health when operating the equipment continue to hinder on-farm practices (Erasmus, 2009). Birds placed into gas stunning units may stand on top of each other, essentially suffocating them, or birds may not be effectively killed and rather just stunned, where they can regain consciousness after removal from chamber (Raj et. al., 2008; Raj et. al., 2000). Therefore, the use of gas as an effective means of on-farm euthanasia may not be practical.

Cervical Dislocation

Cervical dislocation is an approved method for euthanasia on chicken farms, however due to the large size, cervical dislocating turkeys is used for small, younger turkeys (Erasmus, 2009). Cervical dislocation is the separation of the first cervical vertebrae from the skull, where death occurs due to disruption of the blood vessels within the neck causing cerebral ischemia and loss of brain function (Erasmus, 2009; AVMA, 2020). Cervical dislocation is divided into two categories, manual cervical dislocation and mechanical cervical dislocation. Manual dislocation is generally accomplished by stretching the neck until physical separation of the cervical vertebrae from the skull occur (AVMA, 2020). Mechanical cervical dislocation is the crushing of the cervical vertebrae, death is caused by cerebral anoxia or loss of blood supply to the brain (AVMA, 2020). Manual cervical dislocation results in death from cerebral ischemia. The loss of brain function also results in loss of respiration function and cardiac arrest. Cervical dislocation is widely used in euthanasia of small laboratory animals and is successful in inducing rapid insensibilities (Weisbrod et. al., 1984; Erasmus, 2009).

Similar cervical dislocation studies have been done on poultry species, more specifically broiler chickens (Jacobs et. al., 2019). Birds euthanized via mechanical cervical dislocation induce longer times of evoked responses compared to manual cervical dislocation, however both mechanical and manual methods of cervical dislocation do not result immediate insensibilities (Jacobs et. al., 2019). Cervical dislocation methods have a localized effect on the cervical vertebrae and spinal cord and does not directly affect the brain (Erasmus, 2009). In addition, manual cervical dislocation can induce operator fatigue.

Captive Bolt Devices

Captive bolts are defined as controlled and uniform blunt force trauma applied to the skull (AVMA, 2020). Non-penetrating captive bolts create a uniform blunt force trauma to the skull of the animal causing concussion and trauma to the bran leading to immediate unconsciousness and death. Impact should be perpendicular to the skull, cranial to the ears, caudal to the eyes. Captive bolt devices are widely used as a stunning methods of large farm animals prior to slaughter (Erasmus, 2009). Two types of captive bolt devices exist: non-penetrating captive bolts which crush the skull upon impact and does not penetrate the cutaneous layer on the head of the animal and penetrating captive bolts which crushes the skull with penetration to the cutaneous layer. Measures of an effective kill is tested using and electroencephalography (EEG) and is considered the ideal measurement for brain activity (Woolcott et. al., 2018). Electroencephalography is used in research setting and not practical for on-farm testing. Other physical measures of insensibility are tested such as spinal reflexes, cessation of movement, corneal reflexes and pupillary stimulation (Woolcott et. al., 2018). Non penetrating captive bolt devices have been studied and compared on poultry species. Captive bolt devices when used resulted in immediate insensibilities and suppression (Woolcott et. al., 2018). Other studies involving the use of non-penetrating captive bolts on poultry species resulted in similar results including the immediate suppression of the electroencephalography and visual evoked responses (Raj et. al., 2001; Erasmus, 2009).

Captive bolt devices have potential to maintain effective killing with minimal operator fatigue, and these captive bolt devices have shown to be an effective method of euthanasia. However, concerns arise with these uses. Distress with bird restraining prior to firing, failure of device firing, and improper impact location can cause the birds to regain sensibilities (Erasmus, 2009).

Blunt Force Trauma

Blunt force trauma is the use of a blunt instrument to swiftly impact the head of the animal with a force that renders the animal insensible and lead to death via loss of brain function to vital organs (Erasmus, 2009). Similar to cervical dislocation, blunt force trauma is widely popular in the poultry industry, however there are few studies evaluating its effectiveness (Erasmus et. al., 2010a; Erasmus et. al., 2010b). When applied to other species, blunt force trauma is humane if the impact produces hemorrhaging and skull fractures (Erasmus, 2009; Daoust et. al., 2002). The application of this method may have drawbacks coincide such as a strike without enough force to ensure a kill, and inaccurate strikes which may lead to the bird regaining consciousness. Birds that are examined and determined to have been euthanized unsuccessfully by the strike, exsanguination must follow to ensure the bird does not regain sensibility (Daoust et. al., 2002).

The use of blunt force trauma is an approved method for euthanasia of small laboratory animals and young piglets (AVMA, 2020). Blunt force trauma method is inexpensive, and effective when preform properly, however personnel who perform the method report fatigue and loss of efficacy over time (AVMA, 2020). As similar to cervical dislocation, the efficacy of blunt force trauma is dependent of the skill of the operator.

Electrical Stunning

Electrical stunning is almost universally used in the United States to render poultry species unconscious prior to neck cutting at slaughter facilities. Currently there are two methods to electrical stunning. Full body stunning via water bath stunning where an electrical current is passed through the bird’s entire body and head-only stunning which does not require the use of a water bath or shackle lines making them a more practical use for on farm euthanasia (Erasmus, 2009).

The purpose of electrical stunning is to induce insensibility to preform mechanized or human performed neck cutting and to avoid the bird from regaining consciousness and preform wing flapping during bleeding (Raj et. al., 1998). Inducing cardiac arrest is known to have its advantages on a welfare standpoint. If cardiac arrest occurs at stunning, there is no chance of the bird to regain consciousness and the timing of neck cutting becomes less important (Raj et. al, 1998). When electrical stunning is applied to chickens, 99% of the birds died of cardiac arrest with a minimum 148 mA per bird (Gregory and Wotton, 1987). When turkey electrical stunning is preformed, 90%-100% of turkeys achieved cardiac arrest at 198-250 mA per bird (Gregory and Wotton, 1991b). While increasing the instances of cardiac arrest prior to neck cutting may be considered a welfare benefit, there are still many practicalities to those methods. Each stunner requires that each turkey is stunned individually, and 4 out of 11 turkeys maintained visual evoked responses post stunning and neck cutting (Gregory and Wotton, 1991; Raj, 1998). When a bird is improperly stunned, wing flapping may occur causing neck cutting to be less effective and increase the chance that a bird will regain consciousness. In the event that a bird was not effectively electrocuted effectively, an operator will perform physical neck cutting. Neck cutting may also increase biosecurity risks in the event the animal has a disease (Erasmus, 2009).

Brain Damage Resulting From On-Farm Euthanasia

In order to apply effective euthanasia, the method must result in rapid and irreversible insensibility and death (Erasmus, 2009; AVMA, 2020). When applying physical damage to the brain, sections involved in consciousness such as the cerebral cortex and brainstem are critical to induce insensibility and death (Erasmus, 2009).

Erasmus and coworkers (2010) conducted three studies comparing brain damage resulting from captive bolt stunning and cervical dislocation. Birds euthanized via captive bolt devices showed to have significant brain damage, subcutaneous hemorrhaging and extensive skull fractures. Cervical dislocation methods resulted in low instances of subcutaneous hemorrhaging, brain damage or skull fractures. Indicating that birds euthanized with cervical dislocation methods (mechanical and manual) most likely died from cerebral hypoxia and ischemia, while birds euthanized via captive bolt devices resulted in death from directly disrupting brain function (Erasmus et. al., 2010).

Bader and coworkers (2014) examined the systematic macroscopic, radiographic and histopathologic results on turkeys from blunt force trauma followed by manual or cervical dislocation. All birds were reported having sever subdermal hematomas, brain damage and sever lacerations of the macula oblongata. With extensive damage to the cerebral cortex and brainstem, the indication was insensibilities were immediate (Bader et. al., 2014).

Casey-Trott and coworkers (2014) used a non-penetrating captive bolt pistol to euthanize piglets (3-9kg), where post-mortem measurements included skull fracture displacement, brain hemorrhaging and hemorrhage within specific regions responsible for consciousness and vital organ function. The captive bolt device resulted in immediate insensibility in 98.6% of piglets, with severe skull fractures and brain hemorrhaging were found in all piglets (Casey-Trott et. al., 2014). Additionally, greater instances of brain damage and hemorrhaging were observed in piglets within the lower weight class. The author concluded that the non-penetrating captive bolt pistol resulted in death via widespread skull fractures and brain hemorrhages (Casey-Trott et. al., 2014).

Post-mortem studies on captive bolt and cervical dislocation methods of euthanasia indicate immediate insensibility and death when the presence of extensive brain hemorrhages coinciding with skull fractures.

Assessing Insensibility And Death

The EEG is considered the most reliable indicator of insensibility, since it monitors the activity within the cerebral cortex, which will indicate consciousness (Erasmus, 2009). Two main types of frequencies are monitored, the first being the alpha waves which are associated with the relaxed, awake state of consciousness and beta waves are consistent with the active state of consciousness (Hughes, 1982; Erasmus, 200). The EEG transmits different neural characteristics based on sate of consciousness. Wakefulness produces a correlation dimension of 8-10 beta wave, drowsiness transmits 6-8 alpha waves and slow wave sleep emits 46- delta waves (Coenen et. al., 2009). When preforming euthanasia, determining if insensibilities are rapid based on three distinguishable EEG readings. The complete disappearance or disruption of normal patterns, the escalation of abnormal waves and the complete loss of all wave activity (Lopes Da Silva, 1983; Erasmus, 2009).

The use of an electroencephalogram is considered one of the most reliable indicators of insensibility; however, it is limited to the laboratory setting and is not a practical use for on farm euthanasia (Erasmus, 2009). The EEG also does not directly indicate respiration and heat rate measurements, other physical forms of insensibility are used to determine these specific reactions such as brainstem and spinal reflexes (Erasmus, 2009).

There is not a complete list of studies that indicate a correlation between EEG activity and all brainstem/spinal reflexes. While there are several physical parameters to indicate an animal is insensible and brain death has occurred, a few studies have suggested any EEG activity ceased before the loss of any of the brainstem/spinal reflexes (Martin et. al., 2015). Animals have the ability to express actions in response to a stimulus that may be considered painful. These reflexes include the pupillary light reflexes, nictitating membrane response and spinal reflexes (Erasmus et. al., 2010a).

Photo pupillary response reflex is known as a reliable indication of an animal being completely insensible. The reflex is conducted by shining a light source directly onto the eye, where the pupil is observed for constriction and upon removal of the light the pupil returning to a relaxed state (Croft, 1961; Erasmus, 2009; Martin, 2015). A complete successful euthanasia is applied, the pupil will be unresponsive and fixed in a relaxed state. Sometimes the pupil can be locked into a constricted form (pin-prick pupil) similar to the relaxed state, if the pupil does not dilate the animal is completely insensible (Croft, 1961; Larson and Sessler, 2012; Martin, 2015).

The nictitating membrane is a pale colored/translucent membrane that protects the animal from dust and maintains eye moisture. Many species of animals contain the nictitating membrane including reptile, some mammals and poultry. Studies have been conducted and determined the nictitating membrane reflex is an indicator of brain death/insensibilities (Martin, 2015). The reflex is tested by physically invoking the response by touching the eye to stimulate the medial canthus. The reflex will continue until cessation of the reflex. (Martin et. al., 2015) A study was conducted by Sandercock and coworkers (2014) suggested that when EEG readings have reached an isoelectric wave form, nictitating reflex continued which may be an indicator of the bird being anaesthetized prior to death (Sandercock et. al., 2014; Martin et. al., 2015).

An animal is considered conscious when it is in an awake state with the perception of self and environment (Erasmus et. al., 2010b; Hernandez, 2018). Brainstem and spinal reflexes included as practical on-farm measurements include neck muscle tone, jaw tone, pedal reflexes, convulsions, feather erection and gasping. The flexor reflex is used to determine insensibility, which is activated by nociceptors and is assessed by the foot response when pressure is applied to it (Pedal reflex), however the depth of anesthesia cannot be assessed using this method (Altman, 1980; Erasmus, 2009). The gasping reflex occurs when the bird is observed to have an open beak with deep breathing, often irregular (Woocott et. al., 2018).

The gasping response is not associated with respiration but rather the response of the central nervous system (CNS) to hypoxia (Terlouw et. al., 2016; Hernandez, 2018). The neck muscle and jaw tone require the researcher to assess the rigidity of the muscles within the jaw and neck. Following euthanasia, the operator must test the jaw and neck for resistance (Hernandez, 2018). With the absence of resistance indicates the bird is insensible, additionally these two measures will coincide with the bird's loss of posture (Sandercock et. al., 2014). Woolcott et. al. (2018), however observed the presence of the jaw and neck muscle tone in a few turkeys following a successful euthanasia. Application of these methods may also be difficult to perform accurately due to the convulsions and euthanasia methods affecting the neck (Woolcott et. al., 2018; Hernandez, 2018).

Feather erection is also considered a visual method of insensibility. Feather erection is identified by the sudden erection of the feathers (Hernandez, 2018). Erasmus et. al. (2010) noted the sudden erection of feather following euthanasia of turkeys, however, was not consistently recorded. Feather erection may be used as a possible measurement of death. Gerritzen et. al. (2007) utilized the occurrence of feather erection along with established methods to determine death via carbon dioxide in poultry. Additionally, feather erection is associated with cardiac arrest or disrupted blood flow to the heart (Heard, 2000; Erasmus et. al., 2010a; Hernandez, 2018). Convulsions (Clonic) is the sudden wing flapping, kicking and body movements immediately following successful euthanasia (Woocott et al., 2018). Convulsions are characterized by two phases. The clonic phase occurs immediately and involves violent wing flapping kicking and overall body movements, and the tonic phase which occurs post clonic phase and is observed as the complete rigidity of the legs or wings (Erasmus et. al., 2010a; Hernandez, 2018). The absolute absence of convulsions (tonic and clonic) indicate the animal is insensible followed by death which has been confirmed by the EEG (Erasmus et. al., 2010a; Dawson et. al., 2009).

Stress: Blood Chemistry

Fear typically coincides with negative stress; however, fear may be a functional emotion. When an animal encounters a predator, a rapid response is required to avoid death. Adrenal hormones must rapidly activate to provide the body with required metabolic activity (Korte, 2000). There are two pathways that the stress response hormone disperses throughout the body. The sympathetic-adrenalmedullary (SAM) axis and the hypothalmo-pituitary-adtenocortical (HPA) axis (Koolhaas et. al., 1999; Post et. al., 2003). The role of glucocorticoids is as an effector of psychological and physical stress (Post et. al., 2003). This hormone when released in its acute form causes a release of reserved energy to stimulate the "fight or flight" response. After "fight or flight" response, the corticosteroids must act on the body to regain homeostasis. Feedback loops are used as mechanisms for the body to regain homeostasis using the HPA axis. When corticosterone levels have reached peak concentration in the blood, a negative feedback is sent to the hypothalamus to halt the production of corticotropic releasing hormone by blocking the glucocorticoid receptor within the hypothalamus which in return, halts the continued production of adrenocorticotropic hormone and subsequently corticosterone production (Post et. al., 2003; Boonstra, 2004; Lotvedt et. al., 2017). Corticosterone can also have negative effects when subject to long-term stress (Post et. al., 2003). Excessive fear can lead to psychological and potential physical damage. When subject to long term stress, birds have reduced growth performance, immunosuppression and reduced reproductive ability. (Post et. al., 2003; Boonstra, 2004; Lotvedt et. al., 2017).

With the increase in speculation into the process of shackling poultry there is little scientific information about the potential stress and welfare implications (Nicole and Saville-Weeks, 1993; Kannan et. al., 1997). A study was conducted by Sparrey and Kettlewell (1994) that stated closer attention should be noted to birds that appear to be in pain and should be slaughtered immediately to avoid further pain and distress. The intensification of poultry production has come with an increased scrutiny on the stress and welfare from raising the birds until slaughter (Post et al., 2003). Hemsworth et. al. (1994) reported broilers exposed to human handling throughout its life showed a lower corticosterone response than birds who had minimal human handling prior to slaughter (Hemsworth et. al., 1994). Conversely Nicole and Saville-Weeks (1993) reported that the birds with the most fearful responses were the ones exposed to the environmental conditions during transportation and gentle handling during the grow out phase (Nicol and Saville-Weeks, 1993). A study conducted by Kannan and Mench (1997) compared the plasma corticosterone levels of broiler chickens experiencing routine human handling prior to shackling and birds that were not handled. Bird's within the handled treatment were routinely picked up and gently placed back on the ground throughout the grow-out period. While handled birds showed lower stress levels than unhandled birds, they were not significantly different (Kannan and Mench; 1997). While it is known that bird collection, travel and shackling will cause an increase in stress. If there is potential to reduce the stress load, those measures should be introduced.

CHAPTER III

ALTERNATIVE GAS EUTHANASIA OF CHICKENS AND PEKING DUCKS UNDER VARIOUS LED LIGHTING AND STRESS RESPONSE OF BROILERS AND PEKIN DUCKS PRIOR TO SLAUGHTER UNDER VARIOUS LED LIGHTING

Introduction

The poultry industry has developed two types of birds over the years which have distinctive genetic performance traits. The meat broiler chicken has been genetically selected for specifically increased growth potential. Broiler chickens are typically raised straight run, where male and female chickens are raised together (Raj et al., 1995). The laying breeds of poultry are genetically predisposed for maximum egg production, while maintaining minimal body weight gain. The males of laying breeds have little economic value and therefore millions are euthanized as a result (Jaksch, 1981).

Current practices for pre-slaughter of poultry consist of collection, transport ending with shackling the birds in a dimly lit room (Gregory and Bell, 1987). Reduced lighting within the shackling room has been considered to have a calming effect where birds will exhibit a subdued behavior (Gregory and Bell, 1987). However, no research has been done on the wavelength of light in these rooms and its effect on bird stress. Behaviors such as wing flapping, struggling and vocalizations are established indicators of stress and fear (Jones, 1996). There has been a correlation between the bird’s ability to see and the resulting stress and fear. Jones (1996) reported that hooded birds resulted in lower instances of vocalizations, wing flapping and signs of struggling when shackled compared to unhooded birds. As a bird struggles to free itself from the shackles, injuries can occur such as broken wings and bruised muscles which result in lower carcass quality and economic losses. There has been no investigation into stress hormone levels in relation to the light color in the shackling rooms prior to stunning.

Most states have specified methods for humane slaughter of market age farm animals, however male layer chicks are not consumed by humans and therefore have little regulations of welfare-based euthanasia (Raj et al., 1995; Jaksch, 1981). Male culls are euthanized via maceration. According to the AVMA guidelines, maceration is believed to create immediate insensibility and death and is an acceptable means of euthanasia for neonatal chicks (AVMA, 2020). Maceration works by physically fragmenting the brain resulting in immediate death. The ability of the maceration machine allows large numbers of chicks to be destroyed quickly and is safe for workers to operate make it the most common euthanasia method. Some issues may arise from using maceration such as tissues and other biomaterials that are not properly cleaned can lead to a breach in biosecurity (AVMA, 2020).

Alternative methods of euthanasia have been studied, most specifically carbon dioxide gas. According to the AVMA (2020), the use of carbon dioxide is an acceptable method of gaseous euthanasia. Carbon dioxide inhalation causes relatively quick insensibilities and death via anoxia (AVMA, 2020). Carbon dioxide is an odorless, colorless and nonflammable gas used mainly to euthanize small animals and lab animals. From an operator standpoint, carbon dioxide is a preferred method because its ability to mechanically be applied to the birds. Carbon dioxide may induce involuntary wing flapping and other terminal movements, which can be unpleasant to the observer. Since carbon dioxide is odorless gas, there can be health hazards to the operator with exposure to the gas in the event of unmaintained delivery systems (AVMA, 2020). Other alternative methods of gas inhalation include the use of nitrogen gas. Similar to carbon dioxide, nitrogen is tasteless, odorless and nonflammable gas (Gurung et al., 2018). Nitrogen gas comprises 78% of normal atmospheric air. When using nitrogen for euthanasia, the oxygen must be displaced to less than 2% to create anoxic conditions (Gurung et al., 2018; AVMA, 2020). Nitrogen has shown to not be aversive to chickens or turkeys, while hypoxia is also shown to have little aversive affects (AVMA, 2020). When performing gaseous euthanasia on ducklings, special attentions should be considered (Gerritzen et. al., 2006). The concentration levels needed to successfully euthanize ducklings may be required to be higher under the assumption that ducks and other waterfowl are less susceptible to asphyxia and hypoxia (Gerritzen et. al., 2006; Powell et. al., 2004). Ducks contain psychological mechanisms that allow them to withstand hypercapnia, however, Pekin ducks do not dive and belong to a group known as dabblers (Belrose, 1981; Hawkins, 2001; Gerritzen et. al., 2006).

When referring to poultry, visions is considered the dominant sense. Special lighting techniques are required to raise commercial poultry (Prescott et. al., 2001). Birds contain four types of cones, while mammals only contain 3 types (Goldsmith et al., 2006). The extra cone within the eye also contains special oil droplets which gives the birds the ability to see partial ultraviolet wavelengths (Goldsmith et al., 2006). Lighting for poultry production may not fully emit the light spectrum that birds use. Incandescent, fluorescent, LED and daylight fluorescent lights are utilized in production, however only the daylight fluorescent tubes are able to span the wavelength spectrum of poultry (Mohammed et al., 2010). Welfare is associated with an animal’s behavior and should be considered (Parvin et. al., 2013). Aggressiveness and reduced weight gain have been associated when birds are exposed to light intensities ~150 lux, where feather pecking, and fighting have been observed (Prayitno et. al., 1997). Additionally, aggression has been observed in broiler chickens exposed to red light, alternatively blue light resulted in low aggression and activity (Manser, 1996). Campbell and coworkers (2015) studied the effects of different lighting on the production and behavior of white Pekin ducks. Ducks raised under red and white light were not observed to have abnormal behavior and maintained average growth, while blue lighting resulted in reduced body weight and erratic behavior. Blue light was considered not appropriate for raising Pekin ducks (Campbell et. al., 2015). Two timepoints where lighting could affect the welfare of poultry during euthanasia are: 1. Prior to slaughter while being shackled prior to stunning 2. During gas euthanasia at the hatchery of cull or non-desirable chicks.

There has been little research on euthanasia of neonatal chicks or ducklings using gaseous euthanasia in conjunction with lighting conditions. Additionally, with maceration being negatively viewed by the public, the poultry industry is moving towards an alternative method for mass killing of neonatal chicks (Leenstra et al., 2011; Gurung et al, 2018) and determining the optimum environmental conditions for the chambers is paramount. The aim of this study was to determine and compare the efficacy of two gaseous euthanasia methods of neonatal ducks and chickens under five separate LED lighting colors, as well as stress of pre-slaughter of market age broiler chickens and Pekin ducks under five LED lighting colors. Parameters used in this study were the latency to loss of posture and cessation of movement, number of vocalizations, vocalizations per second (V-Time) and concentration of blood corticosterone.

Materials And Methods

Ethical Note

All neonatal ducks and chickens were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching [20] guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2018-0136).

Experimental Design

Two gaseous methods of euthanasia and pre-slaughter stress under five separate lighting conditions were evaluated and compared in this study. Experimental treatments included Carbon dioxide (CO_2) + white light, CO_2 + red light, CO_2 + green light, CO_2 + blue light, CO_2 + absence of light, Nitrogen (N_2) + white light, N_2 + red light, N_2 + green light, N_2 + blue light, N_2 + absence of light. Pre-slaughter treatments consisted of white light, red light, green light, blue light and the absence of light. LED lights were obtained from ONCE Animal-Centric Lighting Systems (Minneapolis, MN), and intensities were set to 10 Lux. Two types of neonatal chicks and ducklings were used. Neonates in this study were day-of-hatch Hyline W-36 male layer chicks, day-of-hatch male broiler chicks and day-of-hatch Pekin ducklings. Neonatal euthanasia consisted of 10 replicate trials on male layers, 10 replicate trials on broiler chicks and 5 replicate trials on ducklings. A total of 10 neonates were used in each treatment group for a total of 100 neonates per trial. Each batch of neonates were contained in a box that maintained a comfortable temperature prior to euthanasia and the experimental euthanasia box was cleaned between chicks. Broiler chickens (42 days of age), and Pekin ducks (35 days of age) were used for pre-slaughter stress measurement. Pre-slaughter experiment consisted of 10 trials using 10 broiler chickens per treatment, and 5 trials using 10 Pekin ducks per treatment for a total of 50 birds per trial. All birds were caught and placed into crates prior to shackling.

Gas Chamber

A specialized gas chamber was purchased from Kent Scientific (Torrington, CT, USA). The gas chamber contained a gas inlet and outlet measuring 15 *mm* length, with the chamber measuring 22.9 *cm* width, 15.2 *cm* height, 15.2 *cm* in diameter with and internal volume of 5.29 liters. The gas chamber was used for all 10 treatments.

Gas tank/Flow meter

A specialized gas flow meter purchased from HZXVOGEN connects to either a nitrogen or carbon dioxide gas tank. Both gas containers were obtained from Praxair Gas Co. (Bryan, TX). The meter maintained a controlled flow rate into the chamber at 16 lpm. The set flow rate inducted CO_2 or N_2 into the chamber completely after 19.84 seconds.

Neonatal Euthanasia Procedure

A group of 10 neonates were exposed to each gas treatment and lighting condition until the cessation of movement. Prior to gassing, each neonate was exposed to the lighting treatment for 10 seconds inside the chamber. The neonates were held in the gas chamber for one additional minute to ensure death. Flow rates for both gasses were set to 16 Lpm, filling the gas chamber with 100% CO_2 or N_2 after 19.84 seconds. Latency to loss of posture and cessation of movement were recorded via Fastime stopwatch (Leicestershire, England). Vocalizations were recorded via tally counter from Tally Counter (Wenatchee, WA). Chicks were held for an additional 5 minutes after cessation of movement and observed for recovery.

Behavioral Observations

Each treatment was videotaped to evaluate the behavioral responses of the chicks using a DVC camcorder. The measured variables included the cessation of movement, latency to loss of posture and vocalizations. Each vocalization by a neonate was counted by a tally clicker until cessation of movement. Each neonate in all treatments presented loss of posture and cessation of movement. The loss of posture was determined when the neonates could not stand upright and have control over the body posture (Coenen et al., 2009; Lambooij, 1999). Neonates were determined to have cessation of movement when convulsions such as leg paddling, muscle twitching, wing flapping and all other visible signs of motion completely ceased (Coenen et al., 2009; Lambooij, 1999). Cessation of movement and latency to loss of posture were recorded as time durations, vocalizations were recorded numerically, and signs of ataxia (failure to maintain body balance, flipping and gasping for air) were noticed however were not quantified. Neonates under the absence of light treatments were observed using a Sony FDRAX33 camcorder (Tokyo, Japan). When the neonate was placed into the gas chamber, the camcorder was turned on. Using the night vision feature neonates were observed and timed until cessation of movement.

Pre-slaughter Procedure

Ten birds were randomly selected and gently shackled in a commercial kill room. Each treatment of birds was then subjected to either red, blue, green, white or absence of light within the kill room. All birds were shackled and exposed to the light treatment for a total of 10 minutes. Each bird was electrically stunned and exsanguinated via neck cutting, trunk blood was then collected in lithium heparin vacutainers. Each vacutainer was then centrifuged to collect blood plasma. A commercially available ELISA kit (Enzo) was used to determine the concentration of corticosterone. Each corticosterone sample was recorded in pg/mL, where each treatment was compared based on picogram concentration.

Statistical Analysis

All data was analyzed using One-Way ANOVA a model of y=treatment. Means determined to be significant was separated further by Fishers LSD Test, with accepted significance of $P < 0.05$.

Results

Male Layer Euthanasia

All neonatal male layer culls were successfully euthanized using both gases under all five light treatments. (Table 1) shows the correlation of both gasses along with each light and the interaction of gas and light. For loss of posture, chicks exposed to N_2 had a longer latency than CO_2 ($P < 0.05$). differences were seen between colors with red and green light showing longer latencies than all other colors, the shortest latency to loss of posture was observed with the absence of light ($P < 0.05$). When comparing the interaction of gas type and light color, nitrogen x red light had the longest latency to loss of posture compared to all other interactions, with all CO_2 x colors resulting in the shortest latency ($P < 0.05$).

Additionally, differences were seen in cessation of movement between gas types, colors, and their interaction ($P < 0.05$). Nitrogen gas showed to have the longest latency in the birds compared to CO_2 ($P < 0.05$). When comparing light colors, the longest latency to cessation was observed in birds with the absence of light, while the shortest latencies were observed in white and blue light ($P < 0.05$). The interaction of gas and light color showed the longest latency with N_2 x absence of light, with the shortest latency was observed in CO_2 x white light ($P < 0.05$).

The latency difference between loss of posture and death showed differences in gas type, light color, and their interaction ($P < 0.05$). Nitrogen showed to have the longest latency between loss of posture and death compared to CO_2 ($P < 0.05$). The absence of light was observed to have the longest latency of all colors, and the shortest latency was seen in white light ($P < 0.05$). When comparing the interaction, N_2 x white light showed to have the longest latency compared to all other interactions, and the shortest latency was found in CO_2 x white light ($P < 0.05$).

There were differences in vocalizations between gas type, where the highest instances were observed with N_2, followed by CO_2 ($P < 0.05$). There were no differences ($P > 0.05$) in number of vocalizations between light color and gas x light color interaction. Additionally, differences in the ratio of vocalizations to cessation of movement (V-Time) were observed between gas type ($P > 0.05$). N_2 was observed to have more vocalizations per second compared to CO_2 ($P < 0.05$). There were no differences found in V-Time between light color and gas x light color interaction ($P > 0.05$).

Table 1. All parameters following euthanasia of neonatal male layer chicks exposed to gas and LED light combinations.

Treatment	Loss of Posture (sec)	Cessation of Movement (sec)	Vocalizations (# of Instances)	Loss of Posture to Death (sec)	V-Time (vocals/death)[1]
Carbon dioxide (CO$_2$)	23.67^a	78.14^a	7.23^a	54.48^a	0.083^a
Nitrogen (N$_2$)	55.41^b	146.01^b	99.09^b	90.59^b	0.673^b
Red (~580-780λ)[2]	40.87^a	111.7^{bc}	57.27	70.84^{bc}	0.297
Blue (~430-480λ)[2]	39.81^{ab}	109.34^c	60.40	69.54^{bc}	0.340
Green(~480-580λ)[2]	40.50^a	113.72^b	58.63	73.22^b	0.303
White (~430-780λ)[2]	39.00^b	108.19^c	58.14	69.19^c	0.445
Dark	37.52^c	117.43^a	58.96	79.91^a	0.246
CO$_2$ x Red	23.49^e	79.72^d	7.44	56.23^d	0.094
CO$_2$ x Blue	23.79^e	78.14^{de}	7.67	54.35^{de}	0.096
CO$_2$ x Green	24.07^e	79.09^{de}	6.93	55.02^{de}	0.090
CO$_2$ x White	23.83^e	74.46^e	8.28	50.63^e	0.144
CO$_2$ x Dark	23.14^e	79.30^{de}	5.81	56.16^d	0.072
N$_2$ x Red	58.24^a	143.68^{bc}	97.06	85.44^c	0.686
N$_2$ x Blue	55.82^{bc}	140.54^c	101.29	84.72^c	0.732
N$_2$ x Green	56.93^{ab}	148.34^b	102.11	91.41^b	0.697
N$_2$ x White	54.17^c	141.93^c	97.21	87.76^{bc}	0.698
N$_2$ x Dark	51.90^d	155.57^a	97.76	103.67^a	0.653
Pooled SEM	0.64	1.19	1.55	0.56	0.011
P Value Gas Main Effect	P = 0.00	P = 0.00	P = 0.00	P = 0.00	P = 0.00
P Value Color Main Effect	P = 0.00	P = 0.00	P = 0.272	P = 0.00	P = 0.197
P Value Interaction	P = 0.00	P = 0.00	P = 0.225	P = 0.00	P = 0.197

$^{a\text{-}e}$ Differing superscripts within column indicate significant differences $P < 0.05$.

[1] V-Time was defined as the ratio of vocalizations until death.

[2] Indicates wavelengths of each light color

Broiler Chick Euthanasia

All neonatal broiler chicks were successfully euthanized using both gas types under all lighting treatments. (Table 2) shows the correlation of both gasses along with each light and the interaction of gas and light. For loss of posture, chicks exposed to N_2 had a longer latency than CO_2 ($P < 0.05$). Loss of posture showed to have the longest latency under red, white, and green light, and the shortest latency was observed under blue and the absence of light ($P < 0.05$). When comparing the interaction of gas x light color, the longest latency was seen under N_2 x red light, N_2 x green and N_2 x white light, while the shortest latency to cessation was observed in all CO_2 x light color treatments ($P < 0.05$).

Differences were seen in the latency to cessation of movement in gas type and light color. N_2 maintained the longest latency compared to CO_2 ($P < 0.05$). The longest latencies to cessation between color treatments were observed in green and absence of light ($P < 0.05$). There were no differences observed between the interaction of gas type x light color ($P > 0.05$).

Differences were observed in latency of loss of posture to death between gas type and color of light. N_2 resulted in longer latency compared to CO_2 ($P < 0.05$). Green and the absence of light resulted in the longest latency compared to all other light colors ($P < 0.05$).

When comparing vocalizations and the ratio of vocalization (V-Time) to loss of poster, differences were seen with N_2 gas resulting in more instances of vocalization and higher V-Time compared to CO_2 ($P < 0.05$). No differences in vocalization and ratio of vocalization to cessation of movement was seen between light color and the interaction of gas type x light color ($P > 0.05$).

Table 2. All parameters following euthanasia of neonatal broiler chicks exposed to gas and LED light combinations.

Treatment	Loss of Posture (sec)	Cessation of Movement (sec)	Vocalizations (# of Instances)	Loss of Posture to Death (sec)	V-Time (vocals/death)[1]
CO_2	24.21^{a}	76.80^{a}	5.17^{a}	52.59^{a}	0.053^{a}
Nitrogen	57.92^{b}	155.85^{b}	129.78^{b}	97.93^{b}	0.629^{b}
Red (~580-780 λ)[2]	42.58^{a}	114.66^{b}	55.73	72.08^{b}	0.257
Blue (~430-480 λ)[2]	38.99^{b}	112.51^{b}	52.44	73.52^{b}	0.303
Green(~480-580 λ)[2]	42.48^{a}	120.83^{a}	47.88	78.35^{a}	0.141
White (~430-780 λ)[2]	41.83^{a}	113.78^{b}	53.80	71.95^{bc}	0.291
Dark	39.47^{b}	119.81^{a}	51.81	80.40^{a}	0.148
CO_2 x Red	23.25^{cd}	75.06	5.16	51.81	0.070
CO_2 x Blue	24.09^{cd}	74.38	5.69	50.29	0.078
CO_2 x Green	23.53^{cd}	79.46	5.52	55.93	0.073
CO_2 x White	24.66^{cd}	75.79	5.66	51.13	0.076
CO_2 x Dark	25.54^{c}	79.31	3.81	53.77	0.048
N_2 x Red	61.91^{a}	154.26	177.26	92.35	1.278
N_2 x Blue	53.89^{b}	150.63	185.18	96.74	1.297
N_2 x Green	61.43^{a}	162.19	84.37	100.76	0.528
N_2 x White	58.99^{a}	151.76	101.94	92.77	0.689
N_2 x Dark	53.39^{b}	160.42	99.8	107.13	0.629
Pooled SEM	0.64	1.40	1.59	1.03	0.081
P Value Gas Main Effect	P = 0.00	P = 0.00	P = 0.00	P = 0.00	P = 0.00
P Value Color Main Effect	P = 0.00	P = 0.00	P = 0.46	P = 0.00	P = 0.10
P Value Interaction	P = 0.00	P = 0.34	P = 0.47	P = 0.05	P = 0.45

$^{a-e}$ Differing superscripts within column indicate significant differences $P < 0.05$

[1] V-Time was defined as the ratio of vocalizations until death.

[2] Indicates wavelengths of each light color

Duckling Euthanasia

All neonatal ducklings were successfully euthanized, results are listed in Table 3. When referring to the latency to loss of posture, CO_2 resulted in a faster latency compared to N_2 ($P < 0.05$). The absence of light resulted in the longest latency to loss of posture compared to all other LED light colors ($P < 0.05$). CO_2 x Green light resulted in the shortest latency to loss of posture compared to all other gas x light color treatments ($P < 0.05$). N_2 x Green, N_2 x Blue light resulted in the longest latency to loss of posture compared to all gas x light color treatments ($P < 0.05$).

The latency to cessation of movement was the longest with N_2 gas compared to CO_2 gas ($P < 0.05$). Differences were observed between colors, with the longest latency was seen in white light, with the shortest latency to cessation of movement was seen in red light ($P < 0.05$). When referring to the gas type x light color, the shortest latencies were found in CO_2 x Red light, CO_2 x Blue light, CO_2 x Green light, and CO_2 x absence of light ($P < 0.05$). The longest latency to cessation of movement was observed in N_2 x Blue light compared to all other gas type x light color treatments ($P < 0.05$).

The differences in latency from loss of posture to cessation of movement was not observed between colors and gas type x light color ($P > 0.05$). The longest latency was found under white light, while the shortest latency was found under green and red light treatments ($P < 0.05$). When comparing gas type x light color, the shortest latency was observed with CO_2 x Red light, while the longest latency was observed in CO_2 x White light treatments ($P < 0.05$).

Instances of vocalizations were the highest under N_2 gas compared to CO_2 gas ($P < 0.05$). The highest instance of vocalizations came from the absence of light treatments, with the lowest instance occurred under red light treatments ($P < 0.05$). Similarly, the lowest instance of vocalizations occurred under CO_2 x Red light compared to all other treatments, while N_2 x absence of light had the highest instance of vocalizations ($P < 0.05$).

The ratio of vocalizations to cessation of movement (V-Time) was seen to be the lowest under CO_2 compared to N_2 treatments ($P < 0.05$). The highest V-Time was seen in white light treatments, while the lowest V-Time was observed under Green, Blue and absence of light treatments ($P < 0.05$). There were no differences observed between the interaction of gas type x light color ($P > 0.05$).

Table 3. All parameters following euthanasia of neonatal Pekin ducklings exposed to gas and LED light combinations.

Treatment	Loss of Posture (sec)	Cessation of Movement (sec)	Vocalizations (# of instances)	Loss of Posture to Death (sec)	V-Time (Vocals/death)[1]
CO_2	20.42^{a}	92.35^{a}	6.29^{a}	71.93	0.069^{b}
Nitrogen	52.15^{b}	123.19^{b}	83.56^{b}	71.04	0.685^{a}
Red (~580-780 λ)[2]	35.98^{a}	101.83^{d}	33.76^{d}	65.85^{c}	0.216^{c}
Blue (~430-480 λ)[2]	37.19^{a}	108.49^{b}	47.10^{b}	71.30^{b}	0.266^{ab}
Green (~480-580 λ)[2]	37.26^{a}	105.67^{c}	46.76^{b}	68.41^{c}	0.340^{a}
White (~430-780 λ)[2]	36.5^{a}	115.2^{a}	43.93^{c}	78.70^{a}	0.223^{bc}
Dark	34.5^{b}	107.67^{bc}	53.08^{a}	73.17^{b}	0.356^{a}
CO_2 x Red	21.26^{de}	85.48^{e}	3.02^{g}	64.22^{e}	0.035
CO_2 x Blue	19.46^{ef}	88.92^{e}	6.88^{ef}	69.46^{cd}	0.079
CO_2 x Green	19.34^{f}	91.40^{e}	9.04^{e}	72.06^{c}	0.099
CO_2 x White	21.36^{d}	105.78^{d}	4.32^{fg}	84.42^{a}	0.041
CO_2 x Dark	20.68^{def}	90.16^{e}	8.22^{e}	69.48^{cd}	0.091
N_2 x Red	50.70^{b}	118.18^{c}	64.50^{d}	67.48^{de}	0.549
N_2 x Blue	54.92^{a}	128.06^{a}	87.32^{b}	73.14^{bc}	0.686
N_2 x Green	55.18^{a}	119.94^{c}	84.48^{bc}	64.76^{e}	0.710
N_2 x White	51.64^{b}	124.62^{b}	83.54^{c}	72.98^{c}	0.674
N_2 x Dark	48.32^{c}	125.18^{ab}	97.94^{a}	76.86^{b}	0.788
Pooled SEM	0.745	0.829	1.799	0.499	0.014
P Value Gas Main Effect	$P = 0.00$	$P = 0.00$	$P = 0.00$	$P = 0.308$	$P = 0$
P Value Color Main Effect	$P = 0.00$	$P = 0.00$	$P = 0.00$	$P = 0.00$	$P = 0$
P Value Interaction	$P = 0.00$	$P = 0.00$	$P = 0.00$	$P = 0.00$	$P > 0.05$

$^{a-g}$ Differing superscripts within column indicate significant differences $P < 0.05$.

[1] V-Time was defined as the ratio of vocalizations until death.

[2] Indicates wavelength of each light color

Corticosterone

All results of average corticosterone levels between treatments are described in Table 4. Differences were observed between light color treatments ($P < 0.05$). The absence of light maintained a higher level of corticosterone compared to all other treatments ($P < 0.05$). The lowest average levels of corticosterone were the result of blue and green treatments, with white and red treatments were intermediates ($P < 0.05$).

All results of average corticosterone levels between treatments are described in Table 4. Differences were observed between light color treatments ($P < 0.05$). The absence of light and blue light maintained a higher level of corticosterone compared to all other treatments ($P < 0.05$). The lowest average levels of corticosterone were the result of green and red light treatments, with white light as an intermediate ($P < 0.05$).

Table 4. Average corticosterone levels from poultry following stunning under different monochromatic lighting.

Species	Pekin Duck	Broiler Chicken
Treatment	Corticosterone pg/mL	
Red (~580-780 λ)^1	18580^b	19045^{ab}
Blue (~430-480 λ)^1	29440^a	17504^b
Green (~480-580 λ)^1	18491^b	17799^b
White (~430-780 λ)^1	24244^{ab}	19645^{ab}
Absence of Light	30795^a	21643^a
Pooled SEM	0.495	3.09
P Value	P = 0.00	P = 0.00

$^{a-e}$ Differing superscripts within column indicate significant differences $P < 0.05$
^{1}Indicates wavelength of each light color

Discussion

Gas Euthanasia

In this current study, alternative euthanasia lighting and gas methods were examined to determine their efficacy. Nitrogen showed to have longer latencies to loss of posture, latency difference from loss of posture and death, and cessation of movement compared to carbon dioxide gas in birds after exposure. Normal atmospheric air consists of 78% N_2 by volume, which indicates animals under normal conditions are exposed to a higher concentration of N_2 and require a substantially higher concentration compared to CO_2. The range of 33% - 36% CO_2 is required to induce unconsciousness in birds, while death was in the range of 80% - 90% (Jaksch, 1981; Raj, 1995).

However, the concentration needed to successfully euthanize ducklings may be required to be higher due to the assumption that ducks and other waterfowl are less susceptible to asphyxia and hypoxia (Gerritzen et. al., 2006; Powell et. al., 2004). Ducks also contain psychological mechanisms that allow them to withstand hypercapnia by holding their breath, however, the Pekin duck does not dive and belongs to an alternative group known as dabblers (Belrose, 1981; Hawkins, 2001; Gerritzen et. al., 2006). While the gas is induced into the chamber, neonates may have been exposed to a lethal amount of CO_2 far quicker compared to N_2 (Raj, 1995). Neonates may have been more susceptible to carbon dioxide at a lower concentration as a result of the anesthetic effect (Raj et. al., 1995). Similar results have been seen in studies comparing CO_2, N_2, and low atmospheric pressure system (LAPS), where CO_2 gas resulted in the shortest latencies to loss of posture and death (Gurung et. al., 2018).

All neonates exposed to carbon dioxide showed to have decreased vocalizations compared to nitrogen gas treatments. Carbon dioxide is known to create a feeling of breathlessness when inhaled (Gurung et al., 2018). Due to the high concentration of the carbon dioxide, the chicks may have become unconscious using CO_2 quicker due to the analgesic effect (Brosnan et al., 2007; Gurung et al., 2018).

When comparing the ratio of vocalizations to cessation of movement, CO_2 resulted in a lower ratio under all neonates, while N_2 resulted in a greater ratio of vocalizations compared to CO_2. As previously stated, CO_2 may have induced unconsciousness quicker than N_2, meaning all neonates were conscious for a longer period of time, thus produced more vocalizations per second. While it may be possible that neonates experience greater pain/fear under CO_2 due to the formation of carbonic acid in the mucous membrane (Brosnan et al., 2007). CO_2 gas may have induced the loss consciousness before the carbonic acid was produced, however it is unlikely (Otsuguro et al., 2007).

When introducing the lighting colors, the absence of light treatment consistently showed to have the shortest latency to loss of posture across all neonates. While the instances of vocalizations resulted in no differences in male layer and broiler culls, the absence of light treatment produced a high number of vocalizations, and ducklings produced the most vocalizations. The increased vocalizations could have been the result of the neonates experiencing an increased amount of fear. A study compared the fear response of broiler chickens hooded and unhooded during shackling. Broilers that were hooded birds resulted in higher instances of vocalizations compared to unhooded birds (Jones, 1996). While it is unclear whether fear has an effect on the latencies to loss of posture or cessation of movement, the increased vocalizations under the absence of light may suggest a greater experience of fear in neonates.

The latency to cessation of movement, and the difference between loss of posture and cessation of movement resulted in mixed results when compared to lighting treatments. Loss of posture has been directly correlated to unconsciousness (Insensible), and therefore the light color may not be correlated to latency to cessation of movement and the latency difference between loss of posture and cessation of movement (Raj et. al., 1990; Sandercock et. al., 2014). This may be the result of the neonate's inability to no longer perceive variations of light color while insensible. Additionally, when comparing gas type x light color to all parameters, the differences observed were largely the result of the two gas types, and not necessary the interactions of gas type x light color.

Corticosterone

While this study only focused on LED lighting, other types of lighting have been previously examined. Hunt (2009) demonstrated birds under LED lights showed significantly lower instances of fighting and feather pecking compared to incandescent and fluorescent lighting. Additionally, studies have suggested that the light intensity controls the majority of the bird's behavior (Senaratna et al., 2012). Chickens exposed to light under 24-hour conditions were associated with elevated stress and disrupted sleep patterns and experienced an increase in leg disorders and metabolic diseases. (Classen et. al., 1994; Gordon, 1994; Parvin et. al., 2013). LED lighting should require further research to determine the welfare implications.

When comparing the corticosterone levels between birds exposed to different light colors, the absence of light resulted in the highest blood concentration in both Pekin ducks and broiler chickens. This may further the argument that under the absence of light birds may experience more fear and stress. Broiler chickens housed under Blue light were observed to have reduce activity. Additionally, bird activity has been correlated to increased aggression including fighting and feather pecking (Archer et. al., 2014). Manser (1996) reported broiler aggression was found to be lowest when exposed to blue light compared to white, red, and green light, and an increase in aggression was observed in broiler chickens exposed to red light.

While blue light may result in lower fear and stress in the broiler chicken, blue light treatment showed to have high corticosterone levels in Peking ducks. This could be correlated to an increase in fear and stress under both the absence of light and blue light. Campbell and coworkers (2015) compared the production parameters as well as behavior in Pekin ducks under various color lighting. Birds raised under blue light had significantly lower body weights and showed to have abnormal behavior with excessive fear (Campbell et. al., 2015). With the knowledge that the absence of vision and blue light increases the amount a fear in poultry, Pekin ducks are experiencing greater stress under these lighting conditions. However, the Pekin duck, which is directly related to the Mallard, may prefer blue-colored objects due to the anatomy and physiology of the retina, and reflect the colors that ducks are most capable of visualizing (Campbell et. al., 2015). A study by Hart and Vorobyev (2005), ducks were observed to have a preponderance of blue wavelengths. Both broiler chickens and Pekin ducks may have an increase in fear/stress based on two types of criteria. Based on the results of this study birds that are unable to perceive their environment, or if they are able to clearly perceive their environment based on certain wavelength acuity will result in greater fear and stress.

Conclusion

Neonates that were subjected to nitrogen took longer to lose posture, cease moving and had higher numbers of vocalizations when compared to all CO_2 treatments. While both nitrogen and carbon dioxide resulted in successful euthanasia, CO_2 should be considered as the more effective means of gaseous euthanasia. Differences in light colors could not definitively determine a quicker death will occur; however, light colors may influence the birds stress and fear. The absence of light showed to have a higher number of vocalizations produced. While it is also not practical, the absence of light should be avoided when considering gaseous euthanasia. Green light showed to have lower instances of vocalizations and may be considered as an alternative lighting when performing gaseous euthanasia. When comparing light treatments on pre-slaughter, while also not practical, the absence of light should not be considered. Blue light and white light both were observed having high concentrations in Pekin ducks, while blue light had lower concentrations in broiler chickens. It may be beneficial and recommended to provide green lighting for poultry pre-slaughter, however light color should be considered based on the species of poultry, and their ability to perceive different wavelengths.

CHAPTER IV

COMPARING VARIOUS EUTHANASIA DEVICES AND METHODS ON 8 AND 12-WEEK-OLD TURKEYS

Introduction

On farm euthanasia methods are a necessity for the prevention of disease outbreak and continuous suffering of injured or sick birds (Sparrey et al., 2014). Culling methods should minimize pain and distress, followed by rapid insensibility and death via loss of respiratory function and cardiac arrest (Woolcott et al., 2018). Euthanasia is defined as the humane killing of an animal in a way that minimizes distress and pain (AVMA, 2020). Birds are routinely euthanized to prevent disease outbreak from spreading or to remove sick or injured birds form the flock. The AVMA (2020) defines death via euthanasia by three separate measures, hypoxia or reduced blood flow to the brain, the physical disruption of total brain function leading to loss of respiratory function and cardiac arrest (AVMA, 2020).

According to the AVMA (2020), penetrative and non-penetrative captive bolts, blunt force trauma, mechanical and manual cervical dislocation, injectable anesthetic including barbiturates and gas inhalation via carbon dioxide, carbon monoxide, nitrogen and argon are approved methods of euthanasia for poultry (AVMA, 2020). Both mechanical and manual cervical dislocation however are limited to small birds (under 3kg) and injectable anesthetics, blunt force trauma are limited for laboratory settings (Woolcott et al., 2018). The two main methods used for routine on farm killing of poultry are non penetrating captive bolt, mechanical and manual cervical dislocation (martin, 2018). Cervical dislocation causes death by cerebral ischemia and damage to the spinal cord and brain (Bader et al., 2014; Martin, 2018). A successful euthanasia for manual cervical dislocation is when the first cervical vertebrae are completely separated and detached from the skull. Mechanical cervical dislocation crushes the cervical vertebrae inducing anoxia or loss of blood flow to the brain. Non penetrating captive bolts cause death by concussive force which disrupts brain function to vital organs causing loss of respiratory function and cardiac arrest (AVMA, 2020). Manual cervical dislocation and blunt force trauma can cause operator fatigue when utilized frequently and has loss of efficacy over time (AVMA, 2020).

When evaluating on-farm killing methods, the loss of sensibility (unconsciousness) and loss of respiratory, brain and heart function are necessary to determine their efficacy (Erasmus et al., 2010a; Erasmus et al., 2010b). One of the most reliable methods of determining brain function is the electroencephalography (EEG). The EEG monitors the activity in the cerebral cortex which is linked to consciousness. Evoked responses monitored by the EEG however are measured within a laboratory and are not practical for on farm killing methods (Erasmus, 2009).

Techniques used to evaluate the efficacy of particular euthanasia method are pupillary light reflex, nictitating membrane reflex and spinal reflexes. The pupillary light reflex and nictitating membrane are considered as an indicator of insensibility. A light is shown into the eye of the live animal will cause the pupil to constrict, when the light is removed the pupil will expand. (Erasmus et al., 2010a; Croft, 1961). The nictitating reflex is a pale colored, semi translucent membrane that protects the animal from getting contaminants on the cornea and maintains eye moisture (Martin, 2018). When assessing insensibility via the nictitating membrane response the eye is physically touched, in which the nictitating membrane will move to cover the cornea in a live animal. Insensibility is considered when the nictitating membrane ceases to respond (Sparrey et al., 1993). Additionally, cessation of convulsions is a reliable method for assessing complete brain failure (Dawson et al., 2009). The flexor reflex is the response of nociceptors activating to physical pressure is applied (Altman, 1980). When there is a complete cessation of the nictitating membrane reflex and the pupil becomes fixed, the blood flow to brain has been constricted leading to brain death (Erasmus, 2009).

There are two types of stress in poultry, eustress which is considered normal psychological stress deemed beneficial to the animal and distress which is correlated to the negative type psychological stress that coincides with pain and fear (Zulkifli and Siegel, 1995; Martin, 2018). When considering on farm euthanasia, the majority of distress comes from handling and restraining poultry. Euthanasia that is considered beneficial for the welfare of the animal is to have as little pain and distress while resulting in immediate and irreversible insensibility and death (Blackmore, 1993).

The objective of this study was to compare the efficacy of four non-penetrating captive bolt devices, the Zephyr- EXL, Turkey Euthanasia Device, Jarvis pneumatic stunner and the experimental crossbow. Additionally, three types of cervical dislocation methods including manual cervical dislocation, Koechner Euthanasia Device and the Broomstick method. Efficacy was based on ante-mortem sings of insensibility and clinical signs of death. It is predicted based on limited reports that captive bolt devices will induce rapid insensibilities and death compared to all forms of cervical dislocation methods.

Materials And Methods

Ethical Note

Turkeys were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching [20] guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2018-0355).

Overview

The study was conducted using Cargill (Minneapolis, MN) turkey hens within two age groups, eight weeks old and twelve weeks old (N = 1,400). This experiment consisted of 7 treatments: Zephyr-EXL (ZEP) Jarvis Pneumatic Stunner (JAR), Experimental Crossbow (CRS), Turkey Euthanasia Device (TED), Koechner Euthanasia Device (KED) from Manual Cervical Dislocation (MAN) and Broomstick Method (BRM). Turkeys were tested on 20 separate experimental days over the course of six months, from four separate grow-out facilities near Texas A&M University.

Captive Bolt Devices

Non-penetrating captive bolt devices (Figure 1) with the exception of the experimental crossbow was attached to a Porter-Cable pancake air compressor (Jackson, TN) with pressure set to 125 Psi. The (A) Zephyr-EXL from Bock Industries (Elkhart, IN), is a pneumatic-powered non-penetrating captive bolt, with a mushroom-shaped head attached to a metal bolt. The (B) Jarvis Pneumatic Stunner from Jarvis Products Co. (Middletown, CT), similar to the ZEP, is a pneumatic-powered non-penetrating captive bolt device. The device powers a metal-alloy cylindrical bolt with a flattened bolt head. The (C) Turkey Euthanasia Device from Bock Industries (Elkhart, IN), is a fuel-powered non-penetrating captive bolt (Hitachi NT65GS), containing a flat, steel bolt head. The (D) Experimental Crossbow from Koechner Mfg. Co. (Tipton, MO), consisted of a modified crossbow (The Burst). The Crossbow contained a steel bolt with a plastic cylindrical flattened bolt head. The Crossbow was powered by a cocking lever. The draw weight of the Crossbow was confirmed to be 36.3 kg.

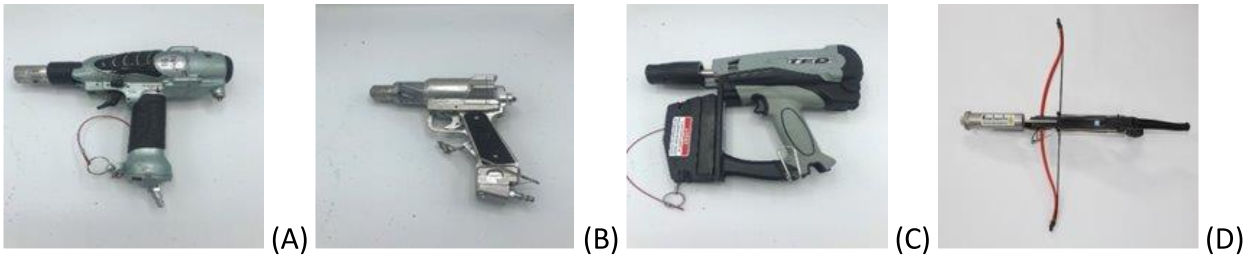

Figure 1. Non-penetrating captive bolt devices (A-D), not connected to external power sources.

The use of each captive bolt device was applied according to previous research protocols (Erasmus, 2009; Woolcott et. al., 2018). One shot was fired on top of the skull between the eyes and center to the ears (Woolcott et. al., 2018). The Zephyr-EXL (A), Jarvis pneumatic stunner (B) and the Turkey Euthanasia device (C) were all powered with 120 PSI across both age groups of turkeys in order to maintain consistency and proper euthanasia.

Cervical Dislocation methods

Manual cervical dislocation method was performed by experienced personnel in accordance to AVMA (2020) guidelines. Two methods of mechanical cervical dislocation were assessed (Figure 2). The (E) Koechner Euthanasia Device from Koechner Mfg. Co. (Tipton, MO), contains 102cm length handle with metal jaw apparatus designed for cervical dislocation. The (F) Broomstick method contained a one-meter length broomstick handle, with two 36cm pool noodle foam inserts on both ends. Pool noodle foam inserts were placed onto the broomstick handles to reduce pain and distress from handling prior to application.

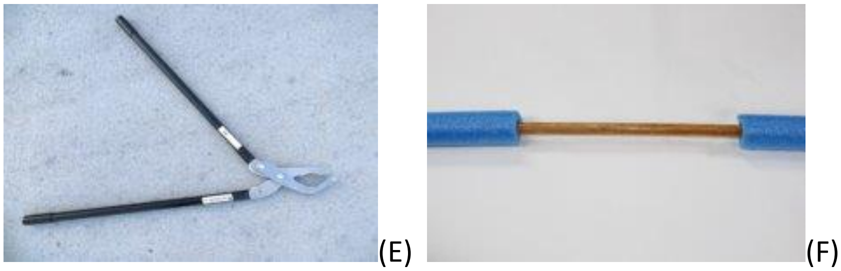

Figure 2. The two mechanical cervical dislocation devices with foam inserts on the broomstick handle.

Euthanasia Procedures

Two turkey age groups were tested in this study, 8 weeks-old and 12 weeks-old. Each captive bolt device was tested on ten birds per trial with a total of ten trials per age group. Each turkey was place on the floor in a sternal recumbent position with the keel on a solid flat table (Martin, 2018). The birds were restrained by one person holding the legs and wings to prevent kicking and wing flapping while recording insensibly measures. Each device was discharged onto the top of the skull. Impacts were cranial to the ears, caudal to the eyes in accordance to previous research (Erasmus et al., 2010a).

When performing cervical dislocation, both mechanical and manual methods required the operator to maintain control of the wings and legs after application until sensibility parameters have ceased. When using manual cervical dislocation, turkey heads were rotated in a cranial to caudle fashion until separation of the vertebrae was completed. Each cervical dislocation method was tested on ten birds per trial with a total of ten trials per age group. When operating the Koechner Euthanasia Device, the bird’s head should rest on a flat surface, with the neck fully stretched. The jaws of the device should line up with the base of the skull. Once jaws of the device and skull are aligned, the handles should be closed rapidly cervically dislocating the vertebrae from the skull. When performing the Broomstick method, the turkey heads should be rested on a flat surface with the broomstick placed on top of the next/base of the skull. Once placement of skull and broomstick are aligned, the operator stepped on the broomstick with both feet while simultaneously pulling upwards with the turkey's legs until dislocation is achieved.

Insensibility Parameters

Immediately following euthanasia via device/method, turkeys were observed for pupillary light reflex, nictitating membrane reflex and cessation of movement (Table 5). All reflexes were checked every 5 s until cessation of movement was confirmed. All insensibility parameters were recorded in time (seconds) using Fastime stopwatch (Leicestershire, England). Time began recording immediately following an attempt until complete cessation of each parameter respectively.

Table 5. Descriptions and procedures performed were carried out according to previous research (woolcott et al., 2018).

Measure	Description	Procedure
Nictitating Membrane Reflex	Ephemeral closure of the nictitating membrane in response to physical stimulation	The medial canthus of the eye was gently touched with fingertip
Pupillary Light Reflex	Constriction of the pupil when exposed to light	Light source from a medical pen was shown directly into the eye
Cessation of Movement (Tonic)	Final episodes of movement including body convulsions and wing flapping	Observing the animal until complete cessation of movement

Post-Mortem Data Collection

Turkey heads and necks were visually inspected for punctures or lacerations immediately following death. Turkey's that presented lacerations or punctures were recorded within the age group and device/method used respectively (N=700). Turkeys euthanized via manual or mechanical cervical dislocation were cut at the base of the neck (last cervical vertebrae), identified and placed into a box for transportation. Turkey necks and skulls were radiographed at the Texas A&M University Veterinary Hospital and analyzed for correct vertebrae separation and if the individual vertebrae were crushed for both 8 weeks-old (N=300) and 12 weeks-old (N=300) turkeys. All radiographed turkey necks/heads that were euthanized via cervical dislocation were viewed and scored as a percentage of occurrence (Table 6).

Table 6. Descriptions for each type of post-mortem data collection, all scores were recorded on a presence or absence basis.

Parameter	Description	Presence	Absence
Laceration/Puncture	Post-mortem observation of cutaneous tearing or penetration	External skin hemorrhage	No visual signs of cutaneous penetration
Location of separation	Inspecting the location of cervical vertebrae separation	The primary cervical vertebrae (C1) was completely detached from skull	Any cervical vertebrae completely separated other than the C1 vertebrae
Separated vertebrae crushed	Separated vertebrae were inspected for sings of damage or crushed	Separated vertebrae that was crushed or broken	Separated vertebrae that was completely intact without being cracked/broken/crushed

Statistical Analysis

All analyses were performed using SAS 9.1 for Windows (SAS Institute Inc., Cary, NC). Because all data were ordinal, they were compared using the Kruskal-Wallis test on the equality of the medians, adjusted for ties. When significant differences were found, the Dwass Steele Critchlow-Fligner method (Hollander and East, 1999) was used to test for all possible comparisons.

Results

Physical Parameters

Differences were seen in the average pressure of captive bolt devices ($P < 0.05$). The TED device resulted in the highest average pressure compared to all devices (Table 7), while the CRS was observed to have the lowest average pressure ($P < 0.05$). Additionally, the average kinetic energy was observed to be the highest with the TED device compared to all other devices, and the CRS showed to have the lowest average kinetic energy ($P < 0.05$).

Table 7. Captive bolt performance. Pressure data was collected using fuji film pressure paper and software. Speed and kinetic energies were conducted using Vicon motion high speed camera.

Device	**Minimum Pressure (psi)**	**Maximum Pressure (psi)**	**Average Pressure (psi)**	**Impact Radius (CM)**	**avg. bolt speed (m/s)**	**Avg. Kinetic energy (joules)**
Zephyr-EXL (ZEP)	20.30^{a}	71.1^{b}	41.35^{c}	0.20^{b}	67.76^{b}	143.16^{b}
Jarvis Stunner (JAR)	18.10^{b}	92.4^{a}	30.70^{d}	0.40^{a}	60.76^{b}	127.71^{b}
Experimental Crossbow (CRS)	18.9^{b}	92.4^{a}	54.24^{b}	0.40^{a}	49.10^{c}	108.93^{c}
Turkey Euthanasia Device (TED)	16.00^{c}	92.8^{a}	66.70^{a}	0.18^{b}	71.02^{a}	958.54^{a}
P Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

All data was analyzed using anova followed by kruskall-wallis, followed by the dwass, steel, critchlow and fligner method where treatment means were significant at $p<0.05$.

$$\boldsymbol{Velocity = \frac{\Delta Distance}{\Delta Time}}$$

$$\boldsymbol{Kinetic\ Energy = 1/2(m)(V^{2})}$$

Jarvis **Zephyr EXL** **TED** **Crossbow**

Figure 3. Captive bolt visual representation of average pressures on fuji film. Green = lower pressure per square inch and red/yellow = higher pressure per square inch.

Insensibility Parameters

Cessation of movement (tonic), nictitating membrane and pupillary reflexes for 8 weeks-old turkeys are presented in Table 8. Turkeys euthanized via captive bolt method did not present nictitating membrane reflexes or pupillary light reflexes. Cervical dislocation methods resulted in prolonged nictitating membrane and pupillary reflexes ($P < 0.05$) compared to what and add means and se.

Average time of nictitating membrane response was highest when using the cervical dislocation methods ($P<0.05$) compared to all captive bolt devices. Within cervical dislocation methods, the KED was indicated with the longest latency of the Nictitating membrane response (119.07 ± 4.05s) than MAN and BRM cervical dislocation ($P<0.05$). Similar results were seen with the pupillary response time, with the cervical dislocation methods resulted in lasting responses ($P<0.05$) while captive bolt devices resulted in immediate cessation. The KED maintained longer latency to cessation of pupillary response (119.59 ± 4.64s) than MAN or BRM methods ($P<0.05$). Movement persisted longest in cervical dislocation methods ($P < 0.05$), with the KED showing the longest latency (184.68 ± 3.33s) until cessation. The CRS and TED captive bolts maintained the shortest latency to cessation of movement ($P<0.05$).

Table 8. Insensibility responses and death for turkeys 8 weeks of age.

Treatment	Nictitating Membrane Response (Seconds)	Pupillary Light Response (Seconds)	Cessation of Movement (seconds)
Koechner Euthanasia Device (KED)	119.07 ± 4.05^{a}	119.59 ± 4.64^{a}	184.68 ± 3.33^{a}
Broomstick (BRM)	73.70 ± 3.39^{b}	72.78 ± 2.12^{b}	168.40 ± 4.28^{ab}
Manual Cervical Dislocation (MAN)	71.95 ± 3.12^{b}	68.08 ± 3.31^{b}	166.97 ± 4.16^{bc}
ZEP	IMED	IMED	148.08 ± 3.86^{cd}
JAR	IMED	IMED	148.29 ± 4.25^{cd}
TED	IMED	IMED	138.53 ± 3.99^{d}
CRS	IMED	IMED	139.23 ± 3.79^{d}

* all euthanasia methods are listed including se values when applicable, fields listed as IMED denotes immediate cessation. Superscripts $^{a\text{-}d}$ indicates a significant difference within its respective section.

Nictitating membrane reflex, pupillary light response and cessation of movement for turkeys 12 weeks-of-age euthanized via captive bolt and cervical dislocation methods are listed in Table 9. Similar results were seen for turkeys 12 weeks-of-age. The average time of nictitating membrane response was highest when using the cervical dislocation methods (P<0.05) applied to the turkeys compared to all captive bolt devices. Comparison within cervical dislocation methods, the KED indicated the longest latency of time before cessation of the Nictitating membrane response (138.28 ± 3.20) compared to MAN and BRM cervical dislocation (P<0.05). Comparing the pupillary response time, the cervical dislocation methods resulted in lasting responses (P<0.05) while captive bolt devices resulted in immediate cessation. The KED maintained longer latency to cessation of pupillary response (140.71 ± 3.25 sec) than MAN or BRM methods (P<0.05). Movement reflexes persisted the longest in cervical dislocation methods (P < 0.05), with the KED showing the longest latency (198.62 ± 3.40) until cessation. The CRS (152.24 ± 5.11sec), JAR (163.28 ± 4.76 sec) and TED (151.81 ± 3.89 sec) captive bolt devices were found to have the shortest latency to cessation of movement compared to all other euthanasia device/methods (P<0.05).

Table 9. Insensibility responses for turkeys 12 weeks of age.

Treatment	Nictitating Membrane Response (Seconds)	Pupillary Light Response (Seconds)	Cessation Of Movement (Seconds)
KED	138.28 ± 3.20^{a}	140.71 ± 3.25^{a}	198.62 ± 3.40^{a}
BRM	111.19 ± 3.61^{b}	117.96 ± 4.91^{b}	191.82 ± 4.69^{ab}
MAN	98.63 ± 3.88^{b}	109.00 ± 4.00^{b}	185.39 ± 4.56^{bc}
ZEP	IMED	IMED	176.90 ± 4.55^{c}
JAR	IMED	IMED	163.28 ± 4.76^{d}
TED	IMED	IMED	151.81 ± 3.89^{d}
CRS	IMED	IMED	152.24 ± 5.11^{d}

* all euthanasia methods are listed including se values when applicable, fields listed as IMED denotes immediate cessation. Superscripts $^{a-d}$ indicates a significant difference within its respective section.

Post-Mortem Data Collection

Turkeys 8 weeks-of-age that were euthanized via cervical dislocation were evaluated and scored (Table 10) based on location of separated vertebrae and whether the separated vertebrae were crush/damaged.

Cutaneous penetration of turkeys 8 weeks-of-age had the highest occurrence (100%) with the TED compared to all other device/methods ($P<0.05$). The JAR (56%), ZEP (59%) had the second highest occurrences among captive bolts ($P<0.05$), at just above 50% of total birds showing signs of cutaneous penetration. CRS had the lowest instances of penetration of all captive bolts ($P<0.05$) with occurrences happening in only 35.35% of turkeys. The KED resulted in the most instances of lacerations ($P<0.05$) among cervical dislocation methods (43.43%). There were no instances where MAN cervical dislocation caused cutaneous lacerations (0%), resulting in MAN cervical dislocation with the lowest instances of all device/methods ($P<0.05$).

Turkeys cervically dislocated manually resulted in the highest instances of C1 vertebrae separation (100%), and lowest instances of damaged/crushed vertebrae (10%) than BRM and KED methods ($P<0.05$). While BRM and KED methods were not significantly different, the KED resulted in the least amount of C1 vertebrae separation (92%), and more instances of damaged/crushed vertebrae.

Table 10. Post-mortem analysis of turkeys 8 weeks of age, all data was recorded as a percentage of occurrence.

Treatment	Separated Vertebrae Crushed (%)	Location Of Separation (%)	Lacerations Or Punctures (%)
KED	92.00^{b}	8.00^{b}	43.43^{bc}
BRM	94.00^{b}	13.00^{b}	11.00^{d}
MAN	10.00^{a}	100.00^{a}	00.00^{e}
ZEP	N/A	N/A	59.00^{b}
JAR	N/A	N/A	56.00^{b}
TED	N/A	N/A	100.00^{a}
CRS	N/A	N/A	35.35^{c}

* Fields listed as n/a denotes data not applicable. Superscripts $^{a\text{-}d}$ indicates a significant difference within its respective section.

Turkeys 12 weeks-of-age that were observed for laceration or punctures after application of device/methods are listed in Table 11, additionally listed are turkeys that were cervically dislocated, radiographed and analyzed for location of separation and crushed/damaged vertebrae.

Cutaneous penetration of turkeys 12 weeks of age resulted with the TED and ZEP maintaining the highest occurrence (100%) compared to all other device/methods ($P<0.05$). The JAR (73%) and CRS (71%) had the lowest occurrences of penetration among captive bolts ($P<0.05$). The KED resulted in the most instances of lacerations ($P<0.05$) among cervical dislocation methods (36%). There were no instances where MAN cervical dislocation caused cutaneous lacerations (0%), resulting in MAN cervical dislocation with the lowest instances of all device/methods ($P<0.05$).

Turkeys cervically dislocated manually resulted in the highest instances of C1 vertebrae separation (100%), and lowest instances of damaged/crushed vertebrae (79%) than BRM and KED methods ($P<0.05$). Following MAN cervical dislocation, BRM maintained the second highest instances of C1 vertebrae separation (29%), however KED resulted in more ($P<0.05$) crushed/damaged vertebrae (95%) than BRM (70%).

Table 11. Post-mortem analysis of turkeys 12 weeks of age, all data was recorded as a percentage of occurrence.

Treatment	Separated Vertebrae Crushed (%)	Location Of Separation (%)	Lacerations Or Punctures (%)
KED	95.00^{c}	5.00^{c}	36.00^{d}
BRM	70.00^{b}	29.00^{b}	3.00^{e}
MAN	21.00^{a}	100.00^{a}	0.00^{e}
ZEP	N/A	N/A	100.00^{a}
JAR	N/A	N/A	73.00^{bc}
TED	N/A	N/A	100.00^{a}
CRS	N/A	N/A	71.00^{c}

* Fields listed as n/a denotes data not applicable. Superscripts $^{a\text{-}d}$ indicates a significant difference within column ($p < 0.05$).

Discussion

This study compared the efficacy of four non-penetrating captive bolt devices and three cervical dislocation methods on two age groups of turkeys to determine if they induced rapid insensibility and death. Each device/method resulted in a successful euthanasia in all turkeys of 8 and 12 weeks-of-age to determine if there was a difference in efficacy at different ages.. Some studies have reported unsuccessful attempts of euthanasia using captive bolt devices (Woolcott et. al., 2018) contrary to what was observed in this current study. When preformed successfully, captive bolt devices will induce traumatic brain injury. Traumatic brain injury physically disrupts regions of the brain that controls vital organ function (Andriessen et. al., 2010). During an unsuccessful attempt, the skull of the animal may have not been penetrated or vital sections of the brain remained untouched. Reasons for failure included loss of air pressure within the device, and inaccurate location of application. It is recommended that operators should inspect air compressors and gas canisters prior to euthanasia attempt (Woolcott et. al., 2018). Captive bolt devices are designed to decrease the need for physical restraints; however, restraint may be needed to reduce injury and increase visual aesthetic when preforming on-farm euthanasia within the poultry barn. Captive bolt devices were observed to have issues with wear and maintenance. Specifically, the CRS, however these observations were not recorded. All attempts with all captive bolt devices resulted in successful euthanasia in this current study.

These studies reported successful euthanasia in all turkeys killed by cervical dislocation, however higher rates of successful euthanasia were observed using manual cervical dislocation than mechanical cervical dislocation (Erasmus et. al., 2010; Woolcott et. al., 2018). When performed correctly, cervical dislocation should sever carotid arteries, jugular veins, and dislocate the C1 cervical vertebrae from the skull causing brain ischemia and loss of brain function to vital organs (Martin, 2016). Other studies have confirmed similar success rates with cervical dislocation (Erasmus et. al., 2010; Martin, 2016) to what was observed in this current study. While cervical dislocation is considered as a preferred option due to its relatively low cost and practicality, some operators may have difficulty performing this method especially on older and larger birds. While the AVMA (2013) lists cervical dislocation as an approved method for birds weighing less than 3kg, not all operators are physically capable of applying the method successfully. All turkeys euthanized via cervical dislocation in this study were restrained by hand, which may be difficult for certain operators.

When comparing the physical parameters of each captive bolt device, the TED resulted in the greatest pressure and kinetic energy compared to all devices ($P < 0.05$). The power source of the device may be the reason for the substantial increase in power compared to the bow string of the CRS and the air pressure of the ZEP and JAR. Additionally, the small bolt head attached to the TED device might have resulted in the higher average pressure. Cutaneous penetration was most present when using captive bolt devices. TED resulted in the most instances of penetration across both age groups. Bolt design may have an impact on cutaneous penetration. The occurrences of penetration were found to be consistent with bolt head design. the ZEP bolt head was designed with a pointed rubber tip, while this increased the depth of brain trauma, it also increased the percentage of cutaneous penetration. The CRS and JAR both have flat bolt heads, leading to a decrease in penetration percentages. The bolt head of the TED may have led to the increase in instances because of its small diameter (19.1mm) and hard steel material. Published studies have confirmed that captive bolts result in greater percentages of penetration, with the Zephyr-EXL (93%) and TED (92%) both resulting in cutaneous penetration (Woolcott et. al., 2018). Additionally, higher instances of skin penetrations may be due to the kinetic energy of the TED device. With an average kinetic energy of 958.54 joules, the TED has the greatest amount of energy per shot and may lead to increased penetration. Higher percentages of penetration were found in turkeys 12 weeks of age. Higher instances of penetration may have occurred due to skull fractures breaking the skin. While younger turkeys have thinner, mailable skulls, older turkeys have more rigid intact skull which can lead to sharp fractures that penetrate the skin (Woolcott et. al., 2018).

Pupillary light and nictitating membrane responses have been used as reliable, practical on-farm euthanasia measures for determining insensibilities and brain death (Martin, 2016; Erasmus, 2009). Sandercock et. al. (2014) reported that cessation of the nictitating reflex and pupillary light response were representative of bird death. An EEG analysis of these measures were confirmed (Sandercock et. al., 2014) and therefore selected as a measure of brain death and proper euthanasia in this study.

These ante-mortem measures demonstrated that captive bolt devices are capable of performing successful euthanasia without the presence of any sensibilities. The absence of pupillary light response and nictitating membrane reflex when using captive bolt devices are consistent to similar studies (Erasmus et. al., 2010; Martin, 2016; Woolcott et. al., 2018). PSI pressures used in this study were slightly higher (120Psi) to create a similar impact to the CRS and TED, in which the impact pressure of the devices is fixed. Compared to other similar studies, PSI pressures were slightly lower at 100-115 PSI (Woolcott et. al., 2018). Which may suggest that greater percussive force caused greater destruction of brain function resulting in immediate brain death.

Cervical dislocation methods when preformed resulted in extended pupillary and nictitating membrane responses. These latencies may be caused by the absence of brain trauma, with damage occurring to carotid arteries and brain stem (Martin, 2016). With death occurring via brain ischemia, consciousness may be overserved for several seconds post application. Studies conducted assessing cervical dislocation methods demonstrated similar results (Martin, 2016; Bandara et. al., 2019). Mechanical cervical dislocation (KED) resulted in increased latency to cessation of the nictitating and pupillary light reflexes. The objective for cervical dislocation is to dislocate the neck at the highest point (C0-C1) and sever carotid arteries, however mechanical cervical dislocation was found to only cause dislocation or disruption of the spinal cord increasing the latency to ischemia and brain death (Martin, 2016).

Cessation of movement which is an indicator of death was observed with all applications of euthanasia. Cessation of movement following the use of captive bolt devices resulted in shorter latencies, which may be due to the severity of brain trauma induced. Woolcott et. al. (2018) demonstrated how certain captive bolt devices induce rapid cessation of movement based on the severity of skull fractures and brain trauma. Turkeys that resulted in a failed euthanasia attempt showed to have less brain and skull destruction (Woolcott et. al., 2018). Older age turkeys (12 weeks) resulted in increased latencies to cessation of movement than the younger counterparts (8 weeks), which may have been the result of greater bone development of the skull (Woolcott et. al., 2018). Additionally, pervious head injuries, device misplacement and operator skill may cause variability to latencies of death.

Cervical dislocation methods resulted in increased latencies to cessation of movement. This may be due to the lack of brain trauma that occurs when applying this method type. MAN cervical dislocation resulted in shorter latency to cessation compared to the KED and BRM methods. Results were similar to other studies, which demonstrated that manual cervical dislocation (twisting, pulling motion) severed both carotid arteries while mechanical cervical dislocation (crushing) severs only one or neither artery (Erasmus et. al., 2009; Martin, 2018; Bandara et. al., 2019). Additionally, movement was observed to have greater latency in the 12 weeks-old turkeys. This may be the result of greater skeleton development, as a birds age increases, the vertebrae may become fused to the base of the skull which will increase the amount of connective tissue within that area (McLeod et. al., 1964; Martin, 2018). The fused vertebrae may have decreased the rate of brain death.

Lacerations on the neck of turkeys were seen highest when using mechanical cervical dislocation, specifically the KED method. The KED was designed to have steel jaws, while durability and ease of use are proponents, they may also lead to a greater percentage of cutaneous tearing. When performing MAN cervical dislocation, the operator can physically "sense" the separation of vertebrae and halt the stretching, while KED and BRM methods the sensation is not felt. Younger turkeys (8 weeks) should to have higher percentages of lacerations, which may be indicative to the size of the vertebrae. With a smaller spinal vertebra, the KED device was forced to increase the amount the lever was closed. The range of motion may have had a greater impact on the percentages of lacerations occurring in young turkeys. Jacobs et. al. (2019) demonstrated similar results with mechanical cervical dislocation. The mechanical cervical dislocation method (KED) resulted in more external skin damage in layer chickens compared to manual cervical dislocation (Jacobs et. al., 2019).

Analysis of the radiographed turkeys resulted with higher percentages of crushed/damaged vertebrae on birds killed via the KED. This is due to the KED method of crushing the vertebra to create the separation. MAN cervical dislocation separated the vertebrae via twisting and stretching, which resulted in a higher percentage of complete separation. Erasmus and coworkers (2010) demonstrated similar results with the Birdizzo, where the device dislocated the turkey's necks by crushing. While some studies have resulted in poor successful euthanasia rates (Erasmus et. al., 2010; Martin, 2018), all turkeys in this study were successfully euthanized. Poor euthanasia rates for mechanical cervical dislocation is reflected in the AVMA (2020), in which mechanical cervical dislocation is not an approved method.

Additionally, mechanical cervical dislocation resulted in fewer instances of C1 vertebra separation. Specific placement of the KED device was required to have C1 separation. The design of the KED device resulted in separation of vertebra further down the spine. When applying the KED device, the proper placement of the head within the jaws was difficult to obtain when operating singularly. Bandara and coworkers (2019) reported similar results with the KED device size not proportional to the turkey size. Mechanical cervical dislocation efficacy may be the result of the size of animal and device model utilized, while manual cervical dislocation efficacy is the direct result of the specific operator’s skill.

Conclusion

All captive bolt devices met the strict requirements for euthanasia success. Based on these results, captive bolt devices are the most reliable form of on-farm euthanasia when preformed properly by trained operators. Captive bolts demonstrated immediate insensibilities while decreasing the latency to cessation of movement (death) compared to mechanical and manual cervical dislocation methods. Additionally, the TED and CRS devices allows an advantage with regards to portability. The CRS resulted in immediate cessation of sensibilities and similar latencies to cessation of movement to captive bolts. The CRS also is advantageous for on-farm use due to the absence of a power source allowing substantial use before required mechanical servicing. The TED also may be overpowered, and its head may be too focused on a small area as it resulted in penetration of the skull making it visually not appealing while still effectively euthanizing the bird. While the CRS is considered in this study the most reliable method, this device is still experimental as well as issues of wear and maintenance should be studied further.

CHAPTER V

COMPARING TWO CAPTIVE BOLT DEVICES ON MARKET AGE PEKIN DUCKS

Introduction

On-farm euthanasia is required for the rapid prevention of disease outbreak from a sick bird, or to reduce further pain and distress of an injured bird (Sparrey et al., 2014; AVMA, 2020). When referring to ducks, there is little research for on-farm euthanasia. Duck euthanasia research focuses mainly on mass depopulation methods due to diseases such as the avian influenza. Control of this type of poultry disease requires surveillance, rapid detection and confinement, followed by depopulation, disposal and complete disinfection of the area (Benson et. al., 2009).

Culling methods should minimize pain and distress, followed by rapid insensibility and death via loss of respiratory function and cardiac arrest (Woolcott et al., 2018). The AVMA (2020) defines death occurring by three separate measures, hypoxia or reduced blood flow to the brain, the physical disruption of total brain function leading to loss of respiratory function and cardiac arrest (AVMA, 2020).

Approved methods of euthanasia for poultry are penetrative and non-penetrative captive bolts, blunt force trauma, mechanical and manual cervical dislocation, injectable anesthetic including barbiturates and gas inhalation via carbon dioxide, carbon monoxide, nitrogen and argon (AVMA, 2020). While both mechanical and manual cervical dislocation methods are approved, they are only approved for smaller poultry (Under 3kg), and injectable anesthetics are limited to laboratory settings and are not practical for on-farm use (Woolcott et al., 2018).

Non-penetrating captive bolt devices are designed to create maximum damage to vital sections of the brain linked to control of organ function (Woolcott et. al., 2018). Additionally, non-penetrating captive bolt devices should result in immediate insensibility without penetrating the surface of the skull, eliminating any biosecurity risks that may arise (Erasmus, 2009; Woolcott et. al., 2018). The use of a non-penetrating captive bolt device will require proper restraint of the animal and correct placement of the device to ensure a proper kill (NFACC, 2016; AVMA, 2020).

To determine the efficacy of on-farm culling methods, the loss of sensibility (unconsciousness) and loss of respiratory, brain and heart function must be evaluated (Erasmus et al., 2010a; Erasmus et al., 2010b). The electroencephalography (EEG) monitors the activity in the cerebral cortex which has been linked to consciousness in animals (Woolcott et. al., 2018). The EEG involves measuring electrical brain activity such as brainstem and spinal reflexes, as well as rhythmic breathing which can be used to determine sensibility (Woolcott et. al., 2018). Additionally, the recordings from the EEG have been used as validation to physical responses apparent in an insensible animal (Erasmus, 2009). The use of the EEG is considered for laboratory use only is not practical for determining consciousness and death for on farm euthanasia (Erasmus, 2009). Loss of pupillary light reflex and nictitating membrane reflex are considered to be a measure in brain death in poultry (Sandercock et. al., 2014). The cessation of movement (convulsions) have coincided with an isoelectric EEG measurement and can be used as a determinant of clinical death (Woolcott et. al., 2018).

To determine if an animal is sensible post-application light is shown into the eye of the animal in which the pupil will constrict and expand as the light is removed. (Erasmus et al., 2010a; Croft, 1961). The nictitating reflex is a pale colored, semi translucent membrane that protects the animal from getting contaminants on the cornea and maintains eye moisture (Martin, 2018). The nictitating membrane response is to touch the surface of the eye, in which the nictitating membrane will attempt to close to protect the cornea. Insensibility is considered when the nictitating membrane ceases to respond (Sparrey et al., 1993). Cessation of convulsions may be considered as clinical death, however the heart my still function irregularly long after brain failure has occurred (Dawson et al., 2009).

The objective of this study was to compare the efficacy of two non-penetrating captive bolt devices, the Zephyr-EXL and the Experimental Crossbow. Efficacy was based on ante-mortem sings of insensibility and clinical signs of death. It is predicted based on reports of similar studies that both captive bolt devices will induce rapid insensibilities and death.

Materials And Methods

Ethical Note

Pekin ducks were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching [20] guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2018-0355).

Overview

This study was conducted using Pekin Ducks acquired from a commercial source. Ducks were raised to 35 days-of-age. Two trials were attempted with 30 ducks per captive bolt for a total of 60 ducks per treatment. Captive bolt devises included the Zephyr-EXL from Bock industries (Elkhart, IN), and the Experimental Crossbow from Koechner Mfg. Co. (Tipton, MO).

Captive Bolt Devices

The Zephyr-EXL was powered by and attached to a Porter-Cable pancake air compressor (Jackson, TN) with pressure set to 125 Psi. Zephyr-EXL (A) is a pneumatic-powered non-penetrating captive bolt, with a mushroom-shaped head attached to a metal bolt. The Experimental Crossbow (B) consisted of a modified crossbow (The Burst). The Crossbow contained a steel bolt with a plastic cylindrical flattened bolt head and is powered by a metallic alloy arm and string (Figure 4.). The device is loaded by plastic cocking lever for ease of use when fired multiple times. The draw weight of the Crossbow was confirmed to be 36.3 kg. The use of each captive bolt device was applied according to previous research protocols (Erasmus, 2009; Woolcott et. al., 2018). One shot was fired on top of the skull between the eyes and center to the ears (Woolcott et. al., 2018).

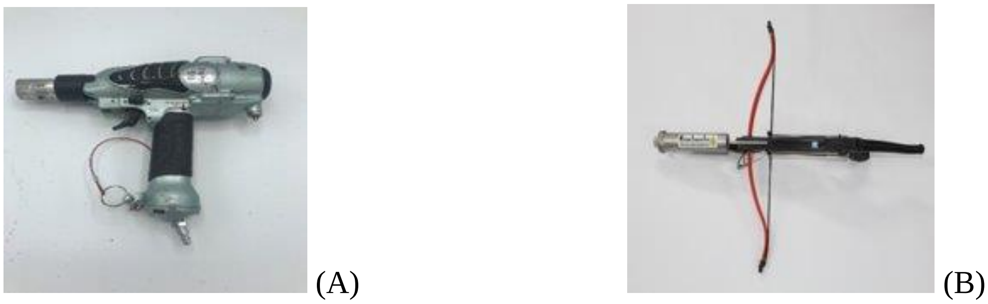

Figure 4. Two non-penetrating captive bolt devices (A-B), not connected to power sources

Euthanasia Procedures

Each duck was place on the floor in a sternal recumbent position with the keel on a solid flat table (Martin et. al., 2018). The birds were restrained by one person holding the legs to prevent kicking while recording insensibly measures. Each device was discharged onto the top of the skull. Impacts were cranial to the ears, caudal to the eyes in accordance of similar turkey research (Erasmus et al., 2010; AVMA, 2020).

Post-Application Measures

Immediately following application of a device, ducks were observed for pupillary light reflex, nictitating membrane reflex and cessation of movement (Table 12). All reflexes were checked every 5 s until cessation of movement was confirmed. All insensibility parameters were recorded in time (seconds) using Fastime stopwatch (Leicestershire, England). Time began recording immediately following an attempt until complete cessation of each parameter respectively. Additionally, following application of device, the cutaneous surface on top of the skull was evaluated for any instances of lacerations or penetration. If a duck showed signs of external skin hemorrhage, the captive bolt device received a score of 1, and absence of any external damage resulted in a score of 0.

Table 12. Descriptions and procedures performed were carried out according to previous research (woolcott et al., 2018).

Measure	Description	Procedure
Nictitating Membrane Reflex	Ephemeral closure of the nictitating membrane in response to physical stimulation	The medial canthus of the eye was gently touched with fingertip
Pupillary Light Reflex	Constriction of the pupil when exposed to light	Light source from a medical pen was shown directly into the eye
Cessation of Movement (Tonic)	Final episodes of movement including body convulsions and wing flapping	Observing the animal until complete cessation of movement
Laceration/Puncture	cutaneous tearing or penetration on top of the skull	Post-mortem observation of cutaneous tearing or penetration

Statistical Analysis

All analyses were performed using SAS 9.1 for Windows (SAS Institute Inc., Cary, NC). Because all data were ordinal, they were compared using the Kruskal-Wallis test on the equality of the medians, adjusted for ties. When significant differences were found, the Dwass Steele Critchlow-Fligner method (Hollander and East, 1999) was used to test for all possible comparisons.

Results

Physical Parameters

Differences were seen (Table 13) in both captive bolt devices ($P < 0.05$). The Experimental Crossbow resulted in the lowest minimum pressure, slower average bolt speed and lowest average kinetic energy compared to the Zephyr-EXL ($P < 0.05$). The Experimental crossbow contains the largest impact radius and highest maximum pressure and average pressure ($P < 0.05$). The Zephyr-EXL resulted in the highest average kinetic energy and average bolt speed ($P < 0.05$).

Table 13. Captive bolt performance. Pressure data was collected using fuji film pressure paper and software. Speed and kinetic energies were conducted using Vicon motion high speed camera.

Device	Minimum Pressure (Psi)	Maximum Pressure (psi)	Average Pressure (psi)	Impact Radius (CM)	avg. bolt speed (m/s)	Avg. Kinetic energy (joules)
Zephyr-EXL	20.30^{a}	71.1^{b}	41.35^{a}	0.20^{b}	67.76^{a}	143.16^{a}
Crossbow	18.9^{b}	92.4^{a}	54.24^{b}	0.40^{a}	49.10^{b}	108.93^{b}
P- Value	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

All data was analyzed using anova followed by kruskall-wallis, followed by the dwass, steel, critchlow and fligner method where treatment means were significant at p<0.05.

$$\boldsymbol{Velocity} = \frac{\Delta \boldsymbol{Distance}}{\Delta \boldsymbol{Time}}$$

$$\boldsymbol{Kinetic\ Energy} = \mathbf{1/2}(\boldsymbol{m})(\boldsymbol{V}^{2})$$

Zephyr-EXL **Experimental Crossbow**

Figure 5. Captive bolt visual representation of average pressures on fuji film. green = lower pressre per sqaure inch and red/yellow = higher pressure per square inch.

Post-Application measures

All ducks were successfully euthanized using both captive bolt devices. All data regarding post-application measurements are listed in Table 14. Both the nictitating membrane response and the pupillary light response resulted in immediate insensibility for both the Crossbow and the Zephyr-EXL. The latency to cessation of movement was higher when using the Zephyr-EXL, however, there was no significant difference (P= 0.333). Differences were observed (P= 0.000) in the percentage of lacerations or punctures in the cutaneous layer on the skull, where the highest occurrence was found in the Zephyr-EXL (85%), compared to the Crossbow (13.3%).

Table 14. Post-application measurements for ducks 35 days-of-age.

Treatment	**Nictitating Membrane Response (Seconds)**	**Pupillary Light Response (Seconds)**	**Cessation of Movement (seconds)**	**Lacerations or punctures (%)**
Zephyr-EXL	IMED	IMED	168.63 ± 0.04	85.0^{a}
Crossbow	IMED	IMED	157.55 ± 7.55	13.3^{b}
P-Value	N/A	N/A	P = 0.333	P < 0.000

* both euthanasia devices are listed including se values, IMED denotes immediate cessation $^{a-b}$ indicates a significant difference.

Discussion

This study compared the efficacy of two non-penetrating captive bolt devices on Pekin ducks to determine if they induce rapid insensibility and death. Several brainstem reflexes have been previously studied and used as practical measurements to determine insensibility and brain death (Erasmus et. al., 2010; Martin et. al., 2016; Woolcott et. al., 2018). Specifically, Sandercock (2014) reported that the nictitating membrane reflex and pupillary reflex directly coincides with brain death (insensible) and was confirmed by use of the EEG. Similar studies to this current study reported the need for proper restraint of the bird to ensure proper application, however, these studies were performed on large turkeys which may require additional restraint (Woolcott et. al., 2018) compared to ducks which can easily be restrained by one person. While captive bolt devices should reduce the need for restraint, it may be necessary to reduce the risks of the bird further injuring itself (AVMA, 2020). All Pekin ducks in this study were restrained by hand and placed on the floor of the barn in sternal recumbency in order to effectively monitor them post-application. This method resulted in little strain to the operator or the bird.

The Zephyr-EXL and the Experimental Crossbow both resulted in immediate cessation of nictitating membrane reflex and pupillary light reflex indicating immediate insensibility. While there are not any previous studies for captive bolt euthanasia on Pekin ducks, the immediate cessations have been observed on other poultry species. Woolcott et. al. (2018) compared to captive bolt devices, the Zephyr-EXL and the Turkey Euthanasia Device, on market age turkeys. Nictitating membrane response and pupillary reflexes were present in few turkeys, with the Zephyr-EXL resulting in 3 of 122 turkeys showing these reflexes (Woolcott et. al., 2018). The current study and these previous studies all demonstrate that using a captive bolt device properly results in immediate insensibility in poultry.

It has been reported by Erasmus coworkers (2010) that determining brain death of the animal can be observed by the EEG, evoked responses, brainstem and spinal reflexes such as the cessation of movement (convulsions), pupillary light reflex and nictitating reflex. While these reflexes have been confirmed on turkeys and chickens, brain death has been linked to the cessation of brain activity when ducks have been killed by means of water-based foam and CO_2 (Caputo et. al., 2012). Additional studies have been completed on mass depopulation of caged laying hens (Gurung et. al., 2018). Where cessation of movement was determined as a conservative approach to brain death and was measured via accelerometer readings (Gurung et. al., 2018). The latencies to cessation of movement (neuromuscular spasms) has been also directly linked to irreversible brain death (Dawson et. al., 2007; Dawson et. al., 2009). Following euthanasia application, convulsions (wing-flapping) will occur followed by a tonic phase which is observed as stiffening of the body with legs and wing outstretched, and possible foot paddling leading to relaxation and death (Raj et. al., 1990; Erasmus et. al., 2010). Woolcott et. al. (2018) reported immediate brain death indicated by the cessation of the nictitating membrane reflex, pupillary reflex and micro/macro indicators of traumatic brain injury. The last movement in turkeys were reported well longer after cessation of eye reflexes and was followed by cardiac arrest (Woolcott et. al., 2018).

All Pekin ducks in this study were effectively euthanized using these two non-penetrating captive bolt devices. These results have been consistent with when performed on turkeys, with a reported 98% success rate when using the Zephyr-EXL (Woolcott et. al., 2018). However, other studies have reported poor success rates. Martin and coworkers (2016) reported 17 of 60 chicks were unsuccessfully euthanized when using a modified penetrating captive bolt device. Most differences with device success have been directly linked to the kinetic force the device delivers or correct placement on the skull, which could be a result of improper restraint (Erasmus et. al., 2010).

Observing any cutaneous hemorrhaging following application could be used as a consideration to observer esthetics and cleanliness. In this study the Zephyr-EXL resulted in far greater instances of external hemorrhaging compared to the Experimental Crossbow. There could be several reasons for the increased penetration. When observing the bolt head design, the Zephyr-EXL has a rubber, mushroom-shaped head attached to a metal bolt. The rounded point on the tip allows for increased penetration into the skull, leading to damage of the brain regions that are responsible for sensibility and vital organ function (Erasmus, 2009). This design may also be the result of increased external hemorrhaging, as penetration is greater, so is the extent of external damage. The Experimental Crossbow contains a plastic, cylindrical bolt head with a flat bolt tip. The surface area of the device may lead to a decreased penetration while still providing sufficient force for successful euthanasia. When comparing average kinetic energy of both captive bolt devices, the Zephyr-EXL contained the highest kinetic energy further indicating an increase in brain damage and external hemorrhaging. While the Experimental Crossbow has a significantly lower kinetic energy, they did not affect latencies to cessation of movement. The increased kinetic energy may only have an effect of external damage and not necessarily increase the rapidity of death. Furthermore, as indicated by the pressure paper results the crossbow not only impacts a larger area but also more uniform pressure across that area. This could also contribute to the greater amount of external hemorrhaging caused by the Zephyr-EXL.

Operator wellbeing and safety should be considered when evaluating euthanasia methods. When evaluating euthanasia methods, safety of personnel, documented emotional effect on observers or operators should be considered (AVMA, 2020). While captive bolt devices have shown that extensive training is not required, personnel who will utilize captive bolt devices should be trained and demonstrate proficiency when applying a technique while being closely observed by a supervisor (AVMA, 2020). Operators should practice on cadavers to instill confidence on placement to ensure a successful kill.

Additionally, device portability should be taken into consideration. While all captive bolt devices require less physical strain, portability may be limited. The Zephyr-EXL is required to have an external air pressure system to power the bolt. While there are portable attachments such as a CO_2 canister, a portable air compressor is considered the best option. With an air canister, there are a limited amount of applications until the pressure is insufficient resulting in an unsuccessful kill. The Experimental Crossbow contained a theoretical unlimited amount of applications. Proper maintenance of all devices should be performed to ensure consistent firings. The Experimental Crossbow does require particular attention to quality of the bow strings, in this study, the bowstrings were observed to be frayed and have damage which can result in an improper kill.

Conclusion

Both the Zephyr-EXL and the Experimental Crossbow meet the criteria for a successful euthanasia and are considered effective and reliable methods for on-farm euthanasia of Pekin ducks. Both devices resulted in immediate cessation of eye reflexes while maintaining similar latencies to cessation of movement. With an increase in cutaneous penetration by the Zephyr-EXL, a biosecurity risk may increase as well as a less desired observer esthetic than the Experimental Crossbow. The Experimental Crossbow may require more maintenance than the Zephyr-EXL, however, the Crossbow does not require and external power source and is more portable. Though the Experimental Crossbow needs further durability testing it may be a solution to some of the drawbacks of powered captive bolt devices.

CHAPTER VI

GENERAL DISCUSSION AND CONCLUSIONS

General Discussion

There has been an increase of awareness on the welfare of animal welfare, more specifically the routine culling of sick or injured birds as well as economically undesired chicks. This research project provided an in depth examination of current, alternative and experimental euthanasia methods on turkeys, Pekin ducks and neonates. Moreover, this is the first scientific evaluation using alternative lighting environments to determine their impact of the fear and stress response during euthanasia.

The first objective of the project was to evaluate the effectiveness of gaseous euthanasia, carbon dioxide (CO_2) and nitrogen (N_2), and whether LED colored lighting impacted the effectiveness and welfare of poultry during euthanasia. In order to evaluate whether LED colored lighting could impact welfare of poultry, it was necessary to understand and determine how birds reacted via behavioral and stress measures. Assessment of corticosterone concentration within blood circulation has been used as a method to evaluate the stress response (Scanes, 2016) and has traditionally been used in poultry. Behavior analysis methods such as number of vocalizations during as stressor have been utilized as an additional test for fear and stress (Chapter 2). Additionally, it is important to consider how poultry perceive certain wavelengths of light. Poultry contain 4 types of cones, with special droplets of oil which improves how they can perceive certain wavelength of light including partial ultraviolet wavelengths (Chapter 2). Special perception of light has shown to have an effect on the general activity of different poultry species. Chickens have increased activity when exposed to red lighting and low activity under blue lighting (Manser, 1996). Pekin ducks have been reported having a preponderance to blue wavelengths leading to an increased fear and stress (Hart and Vorobvev, 2005). Further scientific review showed that when birds are deprived of their visual sense (Absence of light) had an increase in stress and fear response determined by both corticosterone levels and number of vocalizations produced prior to slaughter (Jones, 1998).

In the first experiment, vocalizations produced were high under the absence of light with all neonates. The loss of posture was noted to have quicker latencies when neonates were euthanized under the absence of light. The latencies to cessation of movement and latency from loss of posture to cessation of movement did not show consistent results between light color treatments. This may be the result of the neonate’s inability to no longer perceive variations of light color while insensible. When comparing the corticosterone levels between light colors, the absence of light resulted in the highest blood concentration in both Pekin ducks and broiler chickens. It was also noted that the blue light treatment resulted in high corticosterone in Pekin ducks. Both broiler chickens and Pekin ducks may have an increase in fear and stress based on two types of criteria. Birds that are unable to perceive their environment, or if they are able to clearly perceive their environment based on their certain wavelength acuity will result in greater fear and stress.

To compare effectiveness of gaseous euthanasia, it was required to understand the physical signs of insensibility and death associated with gaseous euthanasia. It has been reported that when oxygen flow to the brain has been deprived, brain function will be disrupted leading to the loss of body posture and ultimately loss of consciousness (Chapter 2). Loss of consciousness has been directly linked to loss of posture in neonates (Chapter 2). To compare both gases, it was important to evaluate how each gas will affect neonates. Carbon dioxide has been known to cause aversive responses in poultry species with the formation of carbonic acid in the mucus membrane (Chapter 2). Additionally, poultry contain specific chemoreceptors which are sensitive to CO_2, resulting in head shaking and gasping prior to unconsciousness (Chapter 2). Poultry do not contain specific receptors to N_2, and therefore do not elicit signs of aversive affects upon exposure. Loss of posture can be considered as a conservative method to evaluate insensibility (Chapter 2). Following loss of consciousness, the brain will cease to act on vital organs resulting in cardiac arrest and death. Without the use of an accelerometer or an EEG, proven physical methods were reviewed and also considered conservative means to determine death (Chapter 2). The cessation of movement which includes the complete cessation of respiratory and other bodily movements are followed by relaxation and death (Chapter 2).

When comparing the efficacy of CO_2 and N_2, the latency to loss of posture, latency to cessation of movement, latency from loss of posture till cessation of movement, vocalizations and V-Time were all higher when neonates were killed under N_2 gas. When considering gas euthanasia for neonates, effectiveness may depend on concentration. Normal atmospheric air consists of 78% N_2 by volume, which indicates animals under normal conditions are exposed to a higher concentration of N_2 and require a substantially higher concentration to induce innocuousness and death. While neonatal chicks are more tolerant to CO_2 during embryogenesis, it has been reported the range of 33% - 36% CO_2 is required to induce unconsciousness, and 80% - 90% range to cause death (Chapter 3). Ducklings also may present an even higher tolerance to anoxic gas. Ducks and other waterfowl are known to be less susceptible to asphyxia and hypoxia (Chapter 3). Ducks contain additional psychological mechanisms that allow them to withstand hypercapnia by holding their breath, however, this is not the case with Pekin ducks, which are grouped as dabblers and do not dive (Belrose, 1981; Hawkins, 2001; Gerritzen et. al., 2006). CO_2 is known to cause a feeling of breathlessness after inhaled which resulted in fewer vocalizations and may not be a result of increased pain and fear. Carbon dioxide is considered to be the better form of gas euthanasia than N_2 with shorter latencies to unconsciousness and death as well as fewer vocalization and V-Time ratio (Chapter 3).

The second objective was to evaluate and compare the efficacy of four non-penetrating captive bolt devices and three methods of cervical dislocation. Two age groups of turkeys were examined using all seven aforementioned methods, in addition two captive bolts were further compared using an experimental crossbow to an effective and widely available Zephyr-EXL captive bolt on market age Pekin ducks. To evaluate the effectiveness of the euthanasia methods, they must be determined when they cause complete insensibility. One of the main proven methods of determining insensibility was the use of the EEG (Chapter 2). However, the EEG could not be considered reliable due to extensive damage occurring on the skull caused by captive bolt application, disrupting electrodes attached to the bird which limits the EEG to laboratory setting only (Chapter 2). There have been multiple studies suggesting physical methods of determining insensibility (Chapter 2). Most notably the eye reflexes such as the nictitating membrane response and the pupillary light reflex, in which the cessation of both reflexes indicates complete insensibility (Chapter 2). To determine death within the turkeys, physical methods were also considered (Chapter 2). One of the main methods of determining death was the observation of complete cessation of movement. As mentioned with gas euthanasia, the complete cessation of tonic and clonic movements are a conservative measurement to determine death in turkeys and ducks (Chapter 2).

In both turkey and ducks’ experiments, captive bolt devices resulted in immediate cessation of both the nictitating membrane response and pupillary light response. The immediate cessation following captive bolt application are indicative of immediate insensibilities. Similar results were seen in turkeys with the latencies to cessation of movements (death) occurring much quicker in captive bolt devices. Cervical dislocation methods resulted in both lasting latencies to eye reflexes and cessation of movement, which is supported further by similar pervious research with chickens (Gregory and Wotton, 1990).

Following determination of latency to loss of posture and cessation of movement, post-mortem measurements were examined. Radiographs of cervically dislocated turkey necks were observed as a means to further compare cervical dislocation methods. Cervical dislocation is accomplished by two methods. Mechanical cervical dislocation is the use of a tool to crush the cervical vertebrae, death is caused by cerebral anoxia or loss of blood supply to the brain. Manual cervical dislocation is the use of personnel to physical stretch the neck of the birds in a cranial to caudle fashion until the complete detachment of the vertebrae from the skull, causing death by cerebral ischemia (Chapter 2). Upon examination of radiographs, turkeys killed by mechanical cervical dislocation showed extensive damage to the vertebrae (crushed) and had very few instances of C1 vertebrae separation or complete separation from the skull. Manual cervical dislocation resulted in complete separation of the C1 vertebrae indicating a correct euthanasia. The further away the vertebrae separation occurs reduces the ability to separate the carotid arteries in the neck subjecting the birds to lasting sensibilities and death (Woolcott et. al., 2018).

The goal of on-farm euthanasia should minimize pain and distress of injured or sick birds. There have been many accredited organizations that have published guidelines and recommendations regarding humane euthanasia. The National Turkey Federation (NTF) advocates for manual cervical dislocation to be the preferred method of euthanasia, however, their weights should be under 3kg. Additionally, the National Chicken Council (NCC) approves of all methods of euthanasia approved by AVMA (2020). The World Organization for Animal Health (OIE) and The Global Animal Partnership (GAP) also recommend manual cervical dislocation of small birds (<3kg). While manual cervical dislocation is considered humane for birds under 3kg, mechanical cervical dislocation is not recommended for euthanasia on poultry of any size (OIE, 2018, GAP, 2017).

An additional post-mortem measurement was recorded in this study. The external hemorrhage following application of method was assessed. Skin penetration was determined as a means to further compare captive bolt devices and cervical dislocation methods. While the penetration or laceration of the methods do not directly affect insensibilities and death, external blood may cause a biosecurity risk considering the on-farm aspect of the experiment. In both experiments, the experimental crossbow resulted in the lowest percentages of penetration to all captive bolt devices. Higher instances of skin penetrations may be due to the differences in kinetic energy of captive bolt devices (Chapter 4). With an average kinetic energy of 108.93 joules, the experimental crossbow had the lowest amount of energy per shot and may have led to the decrease in penetration. While the Zephyr-EXL, TED and Jarvis all had greater average kinetic energies (<127.00), the experimental crossbow maintained enough energy to apply an effective kill of all turkeys and ducks.

Several factors are considered when comparing on-farm euthanasia methods. Additional to minimizing pain and distress, euthanasia methods should be considered safe for operators. With both cervical dislocation methods, there is a level of skill required for a successful application, while captive bolt devices require minimal training. The operator may also experience fatigue when applying manual cervical dislocation to multiple birds which may reduce effectiveness, while captive bolts can be applied multiple times when powered properly.

While it is recommended to restrain birds for both captive bolt and cervical dislocation methods, captive bolt devices require less restraint on the bird providing less distress prior to application. In addition to workers safety, special consideration should apply to captive bolt devices. While durability was not specifically tested in these experiments, proper maintenance of the devices should be consistently monitored. When not properly cleaned and lubricated the devices may not fire properly resulting in an ineffective kill. While misfiring was not observed, the experimental crossbow resulted in bow string replacement due to fraying of the strings. Mobility may also have an effect on captive bolt devices. In order to apply several applications, the zephyr-EXL, turkey euthanasia device and the jarvis stunner all require an external power source. When performing multiple culls, the operator's ability to move throughout the barn may be difficult with these devices. Due to the mechanics of a crossbow, there was not a need to rely on an external power source resulting in the greatest mobility. Captive bolt devices are indicative of a swift and painless euthanasia and should be recommended for on farm euthanasia of poultry. While the experimental crossbow has been considered the most reliable method in these studies, this device is still experimental, and issues of wear and maintenance should be studied further.

Conclusion

With an increase in awareness of routine euthanasia of poultry on-farm, at hatcheries, and in the processing plant, it is important to ensure methods are safe for operators, provide an effective kill, and minimizing pain and distress. In addition, the supplementation of colored LED lights to reduce fear and distress during gaseous euthanasia of neonates and of market age birds at stunning have not been elucidated. Therefore, the first objective of this research was to report the effects of colored LED lights on the stress response and other measures of fear in conjunction with gaseous euthanasia of neonates and also at stunning of market age broilers. The second objective was to evaluate and compare the efficacy of four non-penetrating captive bolt devices and three methods of cervical dislocation, with further investigation and comparison to an experimental crossbow captive bolt to an effective and widely available Zephyr-EXL captive bolt.

Primary findings indicate special perception of light have an effect on the general activity of different poultry species. Birds that are deprived of their visual sense showed to have an increase in stress and showed more indications of fear responses determined by both corticosterone levels and number of vocalizations produced. While Pekin ducks are known to have a preponderance to blue wavelengths, when subjected, ducks resulted in an increase in blood corticosterone concentration and vocalizations produced. Additionally, blue light in chickens and red light in Pekin ducks showed to reduce stress during pre-slaughter, further indicating operators should consider wavelength acuity when implementing LED colored light. Moreover, green LED light did not show to reduce loss of posture and cessation of movement latencies, however, corticosterone concentration was consistently the lowest for both chickens and ducks. While species should be considered, the use of green LED light may be used as the primary lighting for pre-slaughter of chickens and Pekin ducks. While latency to loss of posture was not consistent, the absence of light showed to decrease the latency of this insensibility measurement, suggesting a minor effect on rapidity of unconsciousness. Moreover, carbon dioxide gaseous euthanasia was observed to induce rapid insensibility and death compared to the inert gas nitrogen. Carbon dioxide also reduced the behavior measurements of vocalizations and V-Time in neonates. While these findings are indicative to a decrease in fear and stress, carbon dioxide has been known to cause a feeling of breathlessness as well as the formation of carbonic acid within the mucous membrane which may result in more pain. However, the rapidity of insensibility associated with carbon dioxide may minimize the bird's behavior to the exposure.

Results of turkey and Pekin duck euthanasia suggest lasting sensibilities when cervical dislocation is applied. Following radiograph examination of cervical dislocation methods, turkeys euthanized via mechanical cervical dislocation indicated high percentages of incorrect location and crushing of the cervical vertebrae leading to lasting sensibilities. While manual cervical dislocation is an approved method for birds under 3kg, many organizations do not recommend mechanical cervical dislocation on poultry of any size. Both mechanical and manual cervical dislocation resulted in death in this research, however, the lasting sensibilities and longer latency to cessation of movement further suggest to not recommend mechanical cervical dislocation.

Captive bolt euthanasia results indicate all devices cause immediate insensibility and rapid cessation of movement. The latency to cessation of movement between captive bolts showed minimal differences. However, comparison of the experimental crossbow and the Zephyr-EXL resulted in shorter latency to cessation of movement in Pekin ducks euthanized with the experimental crossbow. While still experimental, results suggest the crossbow is capable of producing immediate insensibility and rapid death comparable to currently available captive both devices.

To further compare captive bolt devices, a post-mortem measurement of external hemorrhages was observed between all methods. External hemorrhages may not impact the efficacy of the device, however, the presence of such could cause concern from a biosecurity and operator perception standpoint. The results suggest the experimental crossbow consistently reduced the percentages of cutaneous penetration among both age groups of turkeys as well as

Pekin ducks. The TED, JAR and ZEP resulted in higher percentages of cutaneous penetration which can be attributed to bolt head design as well as average kinetic energy. The average kinetic energy of these captive bolts was far greater than the experimental crossbow, which causes more external damage. The TED and ZEP also contain small or pointed bolt heads allowing greater penetration into the skull also leading to an increase in external damage.

In addition to assessment of efficacy, euthanasia methods should ensure the safety of the operator and generate a consistent kill. Human error has been a point of concern with cervical dislocation and was further perpetuated in this experiment. Cervical dislocation can be effective when applied to a small number of birds. However, operator exhaustion will occur with cervical dislocation, while exhaustion is not observed with captive bolt devices. Captive bolt devices when properly used, will not cause operator fatigue avoiding human error and maintaining consistent kills. Durability and mobility concerns were observed during this research, where general maintenance and practicality of the devices were noted. With the exception of the experimental crossbow, the JAR and ZEP are required to be connected to a power source. Similar to the TED, the JAR and ZEP can be powered by gas canisters, which aids in mobility, but is limited to a certain number of firings before replacement is necessary. Gas canisters that are not properly recorded and replaced will lead to misfiring and ineffective kills. The experimental crossbow due to the mechanics of a crossbow will allow virtually unlimited number of applications. The experimental crossbow allows the greatest mobility without the need for checking the power source. General maintenance of the captive bolt devices is required to ensure proper working conditions. The TED, ZEP and JAR all required simple general maintenance. While the experimental crossbow required similar maintenance, concerns of wear and tear on the bow strings were observed and may be of some concern with practicality. The bow strings required replacement and constant lubrication to avoid string fraying.

In conclusion, the results from this research project indicate carbon dioxide induced quicker insensibility and death, therefore, CO_2 is considered the most effective and humane method of neonatal gas euthanasia. The absence of light showed to increase the rapidity of unconsciousness during gas euthanasia. Carbon dioxide gas in conjunction with the absence of light is considered the optimal method for euthanatizing neonates. Pre-slaughter lighting further demonstrated when birds that are unable to perceive their environment, or if lighting colors are clearly perceived based on their certain wavelength acuity will result in greater fear and stress. Green lighting allows the birds to perceive their environment while avoiding overstimulation leading to a reduced stress response. Green light was observed to have consistently lower corticosterone concentration for both chickens and Pekin ducks and can be considered to reduce stress prior to slaughter. While all methods of euthanasia resulted in death, captive bolt devices resulted in immediate insensibilities and rapid death. Captive bolt devices are also designed for easy use while remaining durable and are highly recommended for an effective and humane killing method for all poultry species. Moreover, the experimental crossbow is consistent with other captive bolt devices while allowing greater mobility and can be considered as an effective and humane captive bolt, however questions in durability arose and should be further assessed to ensure consistent durability and practicality.

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