

Evaluation of mechanical cervical dislocation, captive bolt, carbon dioxide, and electrical methods for individual on-farm euthanasia of broiler breeders

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ABSTRACT Efficacious euthanasia by applying manual cervical dislocation can be difficult on large and mature poultry. The challenge with using manual cervical dislocation is that the strength required to hold heavy poultry and swiftly apply cervical dislocation can be physically impossible for most people. Therefore, alternative methods of euthanasia are needed for mature and large poultry. Mechanical cervical dislocation using the Koechner Euthanizing Device (**KED**), captive bolt using the Turkey Euthanasia Device (**TED**), carbon dioxide (**CO₂**), and electrical euthanasia were evaluated for use on 65-wk-old broiler breeders at flock termination. Following application of each method, physiological reflexes including the eye nictitating membrane reflex, mouth gaping, and body movement, broken skin, blood loss, kill success, time to cessation of heartbeat, and blood plasma corticosterone levels were assessed. Birds euthanized using the KED had longer response durations for eye nictitating

membrane (91 s) and reflexive mouth gaping (161 s) compared to TED, CO₂, and electrical euthanasia (0–7 s). Body movement durations were also longer for KED (214 s) and TED (209 s) than for CO₂ and electrical euthanasia (0–8 s). The highest percentages of broken skin (93%) and blood loss (96%) were observed for TED, followed by KED (71%, 68%), then CO₂ (0%, 6%) and electrical euthanasia (0%, 3%). No significant differences ($P = 0.1781$) were observed for kill success rates with 98% for KED, 100% for TED, 97% for CO₂, and 100% for electrical euthanasia at 4-min. Time to heartbeat cessation did not differ between KED (659 s), TED (427 s), or CO₂ (583 s) euthanasia methods. No heartbeat was detected following electrical euthanasia. Blood plasma corticosterone levels did not differ between pre-euthanasia or post-euthanasia from any of the methods applied. Based on these results each euthanasia method is acceptable for use with broiler breeders.

Key words: broiler breeder, euthanasia, poultry

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INTRODUCTION

Considerable effort has been devoted to the development of effective, humane methods for euthanizing larger poultry. When euthanasia is the determined option, the technique employed should result in rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, a loss of all brain function and death (AVMA, 2020). Manual cervical dislocation (CD) is considered to be appropriate for euthanasia of poultry but becomes problematic for use with large, older birds such as broiler breeders and turkeys. The most common method for euthanizing poultry on-farm is manual CD,

which is performed by grasping the legs or wings of the bird in one hand then stretching the neck by pulling on the head while applying a backward rotational (rostral-dorsal) force to the head (AVMA, 2020). Alternative methods such as mechanically assisted CD, captive bolt, gas, and electrical euthanasia have been approved by the American Veterinary Medical Association in the Guidelines on Euthanasia for poultry (AVMA, 2020).

Various devices have been developed for mechanical cervical dislocation (MCD) such as the Koechner Euthanizing Device (KED), which is commercially available for poultry euthanasia. The KED is available in several sizes depending on the weight of poultry to be euthanized. Models include a range of sizes from the KED-S for birds up to 1.8 kg, KED-C for birds up to 13.6 kg, KED-T for birds up to 29 kg, and KED-Txl for birds over 29 kg. Both KED and CD have been shown to induce rapid unconsciousness as indicated by immediate onset of clonic/tonic convulsions when used on broilers

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and turkeys, however, the persistence of induced reflexes suggests that the KED method requires additional time to cease brainstem activity and achieve total-brain death (Woolcott et al., 2018b; Hernandez et al., 2019a,b; Jacobs et al., 2019, 2021). At the time this study was conducted, the KED euthanasia devices were thought to cause separation of the cervical vertebrae and were commonly used for mechanical cervical dislocation. However, since this time, the use of the KED-S has been shown to fracture the cervical vertebrae in 3-wk-old turkeys while completing the dislocation of the vertebrae, transection of the spinal cord, and providing effective euthanasia when allowing a 5-min period to attain death. (Woolcott et al., 2018b). In addition, using a KED-T on 8-wk and at 16-wk-old turkey hens resulted in successful euthanasia of all turkeys, however the use of a KED also resulted in fractured cervical vertebrae (Stiewert et al., 2021). Cervical vertebrae dislocation from C1 to C4 all resulted in successful euthanasia, but the vertebrae separation location was skewed cranially ($P = 0.042$) with highest frequency for Occipital–C1 = 53.3% for combined results from broilers, spent hens and turkey hens. However, separation location frequency at occipital–C1 was higher for turkey hens (25 wk) at 83.5% compared to only 52% to 65% for broilers (6 wk) and layers (76 wk) at the same location (Erasmus et al., 2010; Martin et al., 2016). This distribution for vertebrae separation location may in part be due to the anatomy of the connection of the occipital bone of the skull to the atlas and axis vertebrae. The odontoid process extends cranially from the body of the axis (C2) through the vertebral foramen of the atlas (C1) into the occipital – atlas joint (atlanto-occipital) resulting in both vertebrae (C1 and C2) directly and firmly attaching to the base of the occipital bone via ligaments. Differences between bird type, age, and size appear to influence the resulting vertebrae dislocation, but has not been evaluated when used for broiler breeder males and females.

Even though CD is commonly used and considered humane, brain electrical reflex activity (visual evoked potentials) has been calculated to continue for up to 30 s in broilers following application of CD, after subtracting a 70 s delay attributed for anesthesia and continuous mechanical respiratory ventilation (Gregory and Wotton, 1990). However, these birds were under anesthesia and therefore considered unconscious and by definition unable to perceive pain in the cerebral cortex of the brain immediately before, during, or following CD application even with displaying detectable brain electrical reflex activity (Gregory and Wotton, 1990).

Nonpenetrating captive bolt devices, like the Turkey Euthanasia Device (**TED**) are intended to deliver sufficient kinetic energy to the cranium of the skull over the cerebrum to result in immediate insensibility and brain death without penetrating the skin (Woolcott et al., 2018a). The ability of the brain to perceive pain in the cerebral cortex requires functionality of neural pathways in both the cortex and the brain stem. Anesthesia induces unconsciousness and the loss of the ability to perceive pain in the cortex, however the brain stem

maintains its reflex functions as apparent by continued regulation of respiratory ventilation. Total brain death results in the loss of function of both the cerebral cortex and the brain stem resulting in insensibility and response to all external stimuli (Sarbey, 2016), and death from hypoxia in the absence of generation of impulses for respiratory ventilation. Non-penetrative captive bolt euthanasia methods have been shown to be consistently successful at causing immediate insensibility in turkeys (Erasmus et al., 2010; Gibson et al., 2018; Woolcott et al., 2018a; Gibson et al., 2019; Jacobs et al., 2021). In male and female table egg producing chickens (Leghorn and Rock varieties), use of the TED for euthanasia successfully resulted in a rapid onset of death (Bandara et al., 2019b). However, the efficacy of TED for use in broiler breeder euthanasia is not yet well documented and may be complicated by the presence of large combs in mature roosters and hens.

Exposure to gases, such as carbon dioxide (CO_2), leads to insensibility and death by brain hypoxia (Terlouw et al., 2016; AVMA, 2020). The use of CO_2 is an approved method of euthanasia and can be applied for single bird euthanasia either by head only or by placing the bird in a closed container, then gradually increasing the CO_2 concentration over a 4 min time period (Boyal et al., 2020). In addition to individual bird euthanasia for culling, inhaled agents are also approved for on-farm flock depopulation for biosecurity reasons and have been evaluated for use in mass depopulation studies (Kingston, et al., 2005; Turner et al., 2012; AVMA, 2020). However, use of CO_2 for individual broiler breeder euthanasia has not been well documented. In turkeys, CO_2 exposure resulted in quick loss of consciousness based on reflex responses (Jacobs et al., 2021). In some cases, the beginning of CO_2 exposure has been reported to be more aversive (gasping episodes) when applied to poultry than nitrogen or low atmosphere pressure stunning in preparation for slaughter (Gent et al., 2020).

Electrical euthanasia is used to induce immediate unconsciousness and cardiac fibrillation, leading to death from cerebral hypoxia (AVMA, 2020). Electricity is most often considered as a stunning method for slaughter and has less frequently been investigated as a euthanasia method in poultry. When used as a euthanasia method, past studies have utilized electrified water baths with shackle lines which can be impractical for single bird euthanasia, particularly when a bird is injured (Gerritzen et al., 2006b). In the case of slaughter as an alternative for euthanasia, additional factors such as bird shackling and grounding, application of the current through the head (water bath or electrified knife), and meat quality (blood hemorrhages) are considered. However, with euthanasia these considerations are not pertinent and individual bird electrical euthanasia has been demonstrated to be an effective method (Boyal et al., 2020). In turkeys, electric euthanasia resulted in near-immediate loss of consciousness based on very short durations of postapplication reflexes (Jacobs et al., 2021). Electrical euthanasia is advantageous because it does not

chemically contaminate tissues, is economical, and eliminates tonic/clonic convulsions that are common following application of other mechanical euthanasia methods.

Based on work previously conducted using broilers, layers, and turkeys, we hypothesized that KED, TED, CO₂, and electrical euthanasia will all result in death of broiler breeders but latency to loss of physiological responses, bird condition, length of time to death, and corticosterone levels will differ among methods potentially indicating preferred methods. The objectives of this study were to investigate the effectiveness of euthanasia by a single operator with a Koechner euthanizing device (KED), turkey euthanasia device (TED), carbon dioxide (CO₂), and electrical euthanasia in achieving rapid unconsciousness and death in broiler breeders based on physiological reflexes including the nictitating eye membrane reflex, mouth gaping, and body movement, broken skin, blood loss, kill success, time to cessation of heartbeat, and blood plasma corticosterone levels prior to and following euthanasia.

MATERIALS AND METHODS

Birds and Housing

All procedures and all four euthanasia protocols were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC) (PRN #2018-3327). A total of two hundred sixty-two 65-wk-old spent commercial broiler breeders, 131 roosters (4.39 kg) and 131 hens (3.86 kg), were used for this study. Birds were separated by sex into 6 groups of 25 and 4 groups of 28 per pen (2.4 × 4.6 m) with each pen divided into one-third litter, two-third raised wire. All birds had both visual and auditory contact with other birds within pens in the same room. The birds were checked daily at 07:00 and were meal fed 2.72 kg of feed/pen/d. Water was provided ad libitum for all of the 9 d the birds were housed.

Euthanasia Methods

Devices used for each euthanasia method have been described in detail in a previous report by [Boyal et al. \(2020\)](#) and are described briefly below.

Mobile Bird Euthanasia Apparatus In order to perform each method of euthanasia by a single operator, a mobile bird euthanasia apparatus (MBEA) was assembled ([Boyal et al., 2020](#)). Use of the MBEA allowed for the operator to have both hands available to apply each euthanasia method and securing the bird minimized physical activity before each euthanasia method was applied and after each method if convulsions occurred. The MBEA consisted of a cylinder cart with a plastic traffic cone mounted at a 45° angle, which allowed the bird to be inserted into the cone with the head exposed for each method to be applied to the bird. When utilizing the MBEA all 4 methods of euthanasia were able to be successfully performed by a single operator ([Boyal et al., 2020](#)). On the first day, there were 2 operators applying each euthanasia method, one with 30+ years of

experience euthanizing poultry and one trained graduate student. On the second, third, and fourth day each method was applied by the trained graduate student. To account for any potential variation of operator skill across days, each euthanasia method was performed on each day for both male and then female broiler breeders (rotating between methods and sex) resulting in an equal frequency of treatments.

Koechner Euthanizing Device – Mechanical Cervical Dislocation On 4 separate days a total of 59 (30 male and 29 female) broiler breeders were euthanized with the Koechner Euthanizing Device-Chicken (KED) (Model #: 8152605, Clear View Enterprises, LLC., Tontitown, AR). The operator began by placing a broiler breeder into the MBEA with the head extending out of the narrow end of the cone. Then the blades of the KED were slowly brought together until the single blade was in light contact with the base of the skull above the allanto-occipital joint (skull occipital-C1-C2 vertebrae) and the twin blades were ventral behind to the jaw. When the single blade was directly at the base of the skull and perpendicular to the head and neck, the operator quickly brought the handles fully together. A timer was then started, and physiological responses of eye nictitating membrane reflex, reflexive mouth gaping, and body movement were assessed.

Turkey Euthanasia Device – Captive Bolt The turkey euthanasia device (TED) (Bock Industries, Inc. Philipsburg, PA) was applied to a total of 69 (32 male and 37 female) broiler breeders throughout 4 separate days. Euthanasia with the TED was performed as described by the manufacturer. After each bird was inserted into the MBEA, the operator pressed the activator into the locked firing position and placed it on the head of the bird behind the eyes and in front of the ears at the base of the comb (above the cranium covering the cerebrum) and pulled the trigger. Thereafter, a timer was then started, and physiological responses were assessed.

Carbon Dioxide Euthanasia A total of 64 broiler breeders (34 males and 30 females) were euthanized with a single bird carbon dioxide euthanasia system that was developed at Auburn University ([Boyal et al., 2020](#)). Briefly, the CO₂ euthanasia system was constructed using a 20 oz CO₂ gas cylinder connected to a low-pressure regulator. A clear-plastic container was configured by cutting a hole in the container lid and the bottom of a gallon sized plastic bag to accommodate the birds head and neck. The plastic bag was then secured to the lid with tape to prevent gas from escaping. A small hole was drilled into the side of the container where a nylon barbed elbow was inserted and connected to clear PVC tubing, with the other end connected to the low-pressure regulator. A second small hole was drilled into the side of the plastic container where a gas analyzer (F-920 Check It! gas analyzer, 177 Felix Instruments, Camas, WA) was connected to confirm CO₂ concentrations (surrounding the head) below 60% for the first 1 min and above 70% for the remaining 3 min while each of the birds were euthanized by this method as previously described by [Boyal et al. \(2020\)](#).

To begin this method, the CO₂ pressure regulator was adjusted to 5 PSI while the flow valves were closed. After the operator placed the bird into the MBEA the head was inserted through the plastic bag and into the container. The operator gently held the bag closed around the neck of the bird with one hand while slightly opening the flow valve until CO₂ release was audible for 20 s. The CO₂ was maintained below 60% for the first minute. After 1 min had passed the operator gently opened the flow valve again for an additional 30 s in order to increase and maintain CO₂ concentrations above 70% for the remaining time of euthanasia completion within 4 min. At the end of the 4 min process for CO₂ euthanasia, physiological responses of eye nictitating membrane reflex, reflexive mouth gaping, and body movements were assessed. Observations for CO₂ euthanasia were assessed at the end of the euthanasia method (4 min) due to the enclosure of the head in the CO₂ euthanasia unit and because physiological responses of gasping, visually similar to mouth gaping, and body movement would be influenced by to the length of time necessary to induce unconsciousness and subsequent reflexes. For these reasons, physiological response assessments began at the end of method for all treatments including CO₂.

Electrical Euthanasia Electrical euthanasia was performed at 110 volts AC on a total of 70 (35 male and 35 female) broiler breeders. The 110 V AC 60 Hz electrical parameters were selected due to the widespread availability of 110 V AC in common electrical outlets in the United States and many other countries. Euthanizing the birds began by placing each into the MBEA with the head extending out of the end of the cone. A previously developed electrical euthanasia device was used (Boyal et al., 2020). The operator attached one clip to the vent of the bird and the other clip into the mouth and the mandible. Then the operator immediately turned on the switch on the electrical euthanasia device and timer. After 15 s, the switch on the electrical euthanasia device was turned off and the clips were removed. After the clips had been removed, the physiological responses of the bird were assessed.

Physiological Responses

Brief descriptions of physiological measurements are presented in Table 1. After each euthanasia method was

applied, beginning at 15 s post euthanasia and at subsequent 15 s intervals, the presence of the induced eye nictitating membrane reflex, reflexive mouth gaping, and presence of body movement were noted. The nictitating membrane was checked by touching the medial canthus of one eye (Schmidt and Wild, 2014; Jacobs et al., 2019). Reflexive mouth gaping was defined as the bird exhibiting opening and closing movements with the mouth. The duration of all body movements was also recorded including the vent, legs, and tail (Dawson et al., 2007; Jacobs et al., 2019). Recordings stopped when all of the reflexes stopped for a minimum of 60 s (4 consecutive observation periods). The presence or absence of broken skin, blood loss, and kill success, which was defined as only one euthanasia attempt on the bird with no signs of recovery (Martin et al., 2016) were recorded. Recordings were completed on the first day by one operator with 30 + years of poultry experience, one operator who filled out the data sheet, and the operator that applied the method to the bird. Then on the subsequent days reflexes were observed and recorded by one trained operator and the same trained operator who had applied the methods on the first day applied the methods to all birds.

The three broiler breeders that were not successfully euthanized following the first application (as defined as absence of all reflexes at 4 min post application or reinitiation of respiration ventilation movements) of the euthanasia method (2 from KED, one from CO₂) were immediately euthanized by electrical as a secondary method and were not included in the physiological response duration data.

Electrocardiogram Acquisition and Analysis

Electrocardiograms (ECGs) were obtained from 8 male and 7 female broiler breeders that were not utilized for blood collection. Birds that were euthanized by the KED, CO₂, or electrical euthanasia had ECGs recorded on 2 male and 2 female birds each. ECGs were recorded on 2 male and 1 female bird euthanized by CO₂ (signal was lost for the second female). ECGs were acquired using the DSI PhysioTel telemetry system. DSI M01 biopotential 2-lead contacts were modified for external use using lead couplers (Item# 276-0031-001, DSI) attached to a surface ECG kit (Item# 012538-001, DSI). This modification allowed ECG acquisition using disposable,

Table 1. Description of physiological and physical measurements recorded after euthanasia method application on broiler breeders.

Measurements	Description	Assessed
Nictitating membrane	Movement of the third eyelid over the eye after gently tapping the medial corner of the eye	Every 15 s after euthanasia method applied
Reflexive mouth gaping	Repeated opening and closing of the beak	Every 15 s after euthanasia method applied
Body movement	Uncoordinated movement of the body, legs, or wings	Every 15 s after euthanasia method applied
Kill success	One euthanasia attempt being successful and all reflexes absent at 4 min	Post-euthanasia
Broken Skin	Resulting from the euthanasia method being applied	Post-euthanasia
Blood Loss	Resulting from the euthanasia method being applied	Post-euthanasia
Cessation of heartbeat	Cessation of rhythmic heart activity	Electrocardiograms (ECGs)

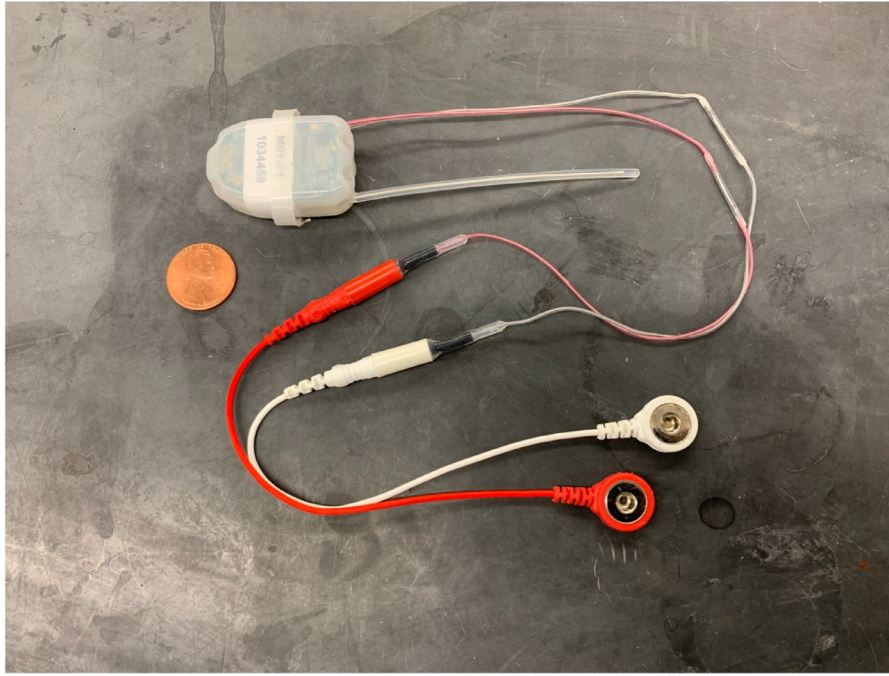


Figure 1. DSI M01 biopotential 2-lead contacts modified for external use using lead couplers attached to a surface ECG kit. This modification allowed ECG acquisition using disposable, self-adhesive external electrodes with the M01 transmitter.

self-adhesive external electrodes (Medgel pediatric foam electrode, Medline, Northfield, IL) with the M01 transmitter (Figure 1). To outfit for recording, immediately prior to use the bird was placed on the breast on a flat surface. The bird handler held both legs extended-out in one hand while keeping the bird calm. A second person then lifted one wing and placed a foam electrode connected to one lead on the skin directly under the wing and on the body, followed by the second lead on the other side of the bird. It was not necessary to remove feathers due to the natural absence of feathers on broiler breeders in this area. Following electrode application, excess lead wire length and the transmitter were clipped to feathers on the bird's back to prevent accidental removal during euthanasia. Once the bird was outfitted, the presence of a strong ECG signal was visualized in real time using the accompanying DSI Ponemah software, which was also used for data acquisition. Determination of time to cessation of heartbeat was defined as no rhythmic heart activity detected.

Blood Corticosterone Analysis

Blood samples were collected from birds both prior to and following euthanasia. Pre-euthanasia blood samples (2–5 mL) were collected and analyzed from a total of 13 broiler breeders. Post-euthanasia blood samples were collected from a total of 84 birds (22 KED, 20 TED, 19 CO₂, and 24 electrical euthanasia), similarly as described by Benson et al. (2007) and Vizzier-Thaxton et al. (2010). Pre-euthanasia samples were taken from the brachial vein and post-euthanasia samples were taken from the heart, which required 2 to 5 min per bird to

collect. Syringe needles were flushed with lithium heparin to avoid blood clotting immediately prior to blood sample collection. Post-euthanasia blood was collected through a cardiac puncture after euthanasia was complete and the bird was confirmed dead. After the abdominal cavity was opened, a syringe needle was inserted into a heart ventricle and 2 to 3 mL of blood was collected. After blood was collected, it was placed in heparinized tubes and placed on ice. The tubes were then centrifuged (Model #: dpr-6000, Dameron/IEC Division, San Diego, CA) at $1559 \times g$ at 5°C for 15 min. Then the blood plasma from the centrifuged blood sample was transferred to a new set of tubes and stored at –20°C. Plasma corticosterone was assessed using a commercially available ELISA kit following the manufacturer's protocol (Cayman Chemical Company Inc., Model #: 501320). Samples were assayed in triplicate and 2 or 3 out of 3 replicates were selected to achieve an intra-assay CV below 15% (intra-assay CV average was 7.8%).

Statistical Analysis

To account for potential difference in observers of physiological responses, the main effect of day was analyzed using the General Linear Models procedure. There was no significant effect of day for nictitating membrane reflex ($P = 0.4000$), reflexive mouth gaping ($P = 0.7174$), or body movement ($P = 0.9462$). Because there was no main effect of day, data from each euthanasia day were combined. Duration of nictitating membrane, reflexive mouth gaping, and body movement physical responses following euthanasia and blood plasma corticosterone levels were analyzed for main

effects of euthanasia method and bird sex as well as method by sex interactions using the General Linear Models procedure with means separated by Tukey's HSD test using the SAS Studio, release 3.8 Enterprise Edition. Latency to heartbeat cessation following euthanasia was analyzed for the main effect of euthanasia method using the General Linear Models procedure with means separated by Tukey's HSD test. Percentage of broken skin, blood loss, and kill success by euthanasia method were analyzed using Chi-square or Fisher's Exact test with significance at $P \leq 0.05$.

RESULTS

Physiological responses of induced nictitating membrane reflex, reflexive mouth gaping, and body movement duration following each euthanasia method are presented in Table 2. Significant main effects were found for bird sex and euthanasia method, as well as sex by method interactions. Therefore, data were analyzed separately for male and female birds. Nictitating membrane reflex duration following KED euthanasia, 111 ± 18 s for males and 70 ± 9 s for females, was significantly longer than following TED, CO₂, and electrical euthanasia (≤ 7 s $P < 0.0001$) indicating that for the vast majority this reflex was absent at the first 15 s observation post application. A similar trend was observed for reflexive mouth gaping duration following KED euthanasia with 216 ± 24 s for males and 105 ± 9 s for females, which was longer than following TED, CO₂, and electrical euthanasia (≤ 7 s, $P < 0.0001$). Durations of body movements for males were similar for KED and TED ($283 \pm$

20 s and 276 ± 19 s) which were longer than CO₂ and electrical euthanasia (≤ 8 s, $P < 0.0001$). Similarly, female duration of body movements was similar for KED and TED (145 ± 8 s and 142 ± 11 s) which were longer than CO₂ and electrical euthanasia (≤ 8 s, $P < 0.0001$). When KED was used for euthanasia, male broiler breeders had a significantly longer duration of nictitating membrane reflex, reflexive mouth gaping, and body movements when compared to female broiler breeders ($P \leq 0.0489$). When TED was used for euthanasia, male broiler breeders had a significantly longer duration of only body movements when compared to female broiler breeders ($P < 0.0001$).

Physical and physiological assessments of broken skin, blood loss, kill success, and heartbeat cessation are presented in Table 3. The broiler breeders euthanized with TED had a higher frequency of broken skin (93%) than KED (71%) while application of CO₂ and electrical euthanasia (0%, 0%) led to no broken skin. Application of TED also resulted the highest frequency of blood loss (96%) followed by KED (68%), followed by CO₂ and electrical euthanasia (6%, 3%), which were not different. No differences were observed in kill success between the four euthanasia methods with 100% success for TED and electrical euthanasia followed by KED at 98% and CO₂ at 97%.

Time to heartbeat cessation did not differ between the KED (659 ± 68 s), TED (427 ± 87 s), and CO₂ (583 ± 76 s) euthanasia methods ($P = 0.1739$). Heartbeat cessation following electrical euthanasia was significantly shorter compared to the other three methods due to the lack of a detectable heartbeat following euthanasia ($P < 0.0001$). When the main effect of sex

Table 2. Physiological response duration following application of mechanical cervical dislocation, captive bolt, carbon dioxide, or electrical euthanasia method application on broiler breeders.

Euthanasia method	Nictitating membrane (s)			Reflexive mouth gaping (s)			Body movement (s)		
	M	F	P value	M	F	P value	M	F	P value
Koechner Euthanizing Device (KED)	$111 \pm 18^{A,Y}$ n = 29	$70 \pm 9^{A,Z}$ n = 29	0.0489	$216 \pm 24^{A,Y}$ n = 29	$105 \pm 9^{A,Z}$ n = 29	<0.0001	$283 \pm 20^{A,Y}$ n = 29	$145 \pm 8^{A,Z}$ n = 29	<0.0001
Turkey Euthanasia Device (TED)	0 ± 0^B n = 32	7 ± 1^B n = 37	0.7059	0 ± 0^B n = 32	7 ± 4^B n = 37	0.0846	$276 \pm 19^{A,Y}$ n = 32	$142 \pm 11^{A,Z}$ n = 37	<0.0001
Carbon Dioxide (CO ₂)	0 ± 0^B n = 33	0 ± 0^B n = 29	- ¹	0 ± 0^B n = 33	0 ± 0^B n = 29	-	1 ± 1^B n = 33	8 ± 7^B n = 29	0.2895
Electrical Euthanasia	0 ± 0^B n = 35	0 ± 0^B n = 35	-	0 ± 0^B n = 35	0 ± 0^B n = 35	-	8 ± 7^B n = 35	0 ± 0^B n = 35	0.2646
P value	<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001	

^{A-B}Values within a column with different superscripts are significantly different ($P \leq 0.05$).

^{Y-Z}Values within a row and within a response type with different superscripts are significantly different ($P \leq 0.05$).

¹No P value was calculated due to lack of response and lack of error.

Table 3. Physiological conditions assessed following application of mechanical cervical dislocation, captive bolt, carbon dioxide, or electrical euthanasia method application on male and female broiler breeders.

Euthanasia method	Broken skin (%)	Blood loss (%)	Kill success (%)	n	Heartbeat cessation (s)	n
Koechner Euthanizing Device (KED)	71 ^B	68 ^B	98	59	659 ± 68^A	4
Turkey Euthanasia Device (TED)	93 ^A	96 ^A	100	69	427 ± 87^A	3
Carbon Dioxide (CO ₂)	0 ^C	6 ^C	97	64	583 ± 76^A	4
Electrical Euthanasia	0 ^C	3 ^C	100	70	0 ± 0^B	4
P value	<0.0001	<0.0001	0.1781		<0.0001	

^{A-C}Values within a column with different superscripts are significantly different ($P \leq 0.05$).

Table 4. Blood plasma corticosterone levels of male and female broiler breeders prior to and following application of mechanical cervical dislocation, captive bolt, carbon dioxide, or electrical euthanasia methods.

	Corticosterone (ng/mL)		P value
	Male	Female	
Pre-euthanasia	17.01 ± 3.73 n = 8	3.39 ± 1.25 n = 5	0.0179
Post-euthanasia			
Koechner Euthanizing Device (KED)	22.10 ± 4.31 n = 11	16.82 ± 3.56 n = 10	0.3630
Turkey Euthanasia Device (TED)	25.04 ± 7.57 n = 9	7.69 ± 2.35 n = 11	0.0286
Carbon Dioxide (CO ₂)	13.07 ± 4.27 n = 11	10.01 ± 4.16 n = 8	0.6239
Electrical Euthanasia	14.67 ± 2.20 n = 13	5.51 ± 2.34 n = 11	0.0090
P value	0.2165	0.0574	

was analyzed, without electrical euthanasia included, there was no difference between males and females (582 and 553 s, $P = 0.7964$) and there was no significant interaction between sex and euthanasia method ($P = 0.5082$).

Blood plasma corticosterone levels prior to euthanasia were significantly impacted by bird sex ($P = 0.0179$) and were consistently higher for males (Table 4). Blood plasma corticosterone levels prior to application of euthanasia methods were 17.01 ± 3.73 ng/mL for males and 3.39 ± 1.25 ng/mL for females. Following euthanasia, corticosterone levels were significantly impacted by bird sex ($P = 0.0035$), but not by euthanasia method ($P = 0.0626$) and did not have sex by euthanasia method interactions ($P = 0.3145$). No significant differences were observed between euthanasia methods for males (13.07–25.04 ng/mL) or females (5.51–16.82 ng/mL). When birds were euthanized by TED or electrical euthanasia, males had higher levels of post-euthanasia corticosterone (25.04 ± 7.57 ng/mL for TED, 14.67 ± 2.20 ng/mL for electrical) than females (7.69 ± 2.35 ng/mL for TED, 5.51 ± 2.34 ng/mL for electrical). For birds euthanized by KED or CO₂ there were no differences between males and females.

DISCUSSION

The goal of this work was to observe and evaluate the physiological and physical differences following four euthanasia methods used on spent broiler breeders. Each method was evaluated based on physiological responses (duration of the induced nictitating reflex, reflexive mouth gaping, and body movement), physical condition (kill success, blood loss, and broken skin), time to cessation of heartbeat, and blood plasma corticosterone.

Physiological Responses and Condition

Physiological responses of eye nictitating membrane reflex, mouth gaping, and musculoskeletal movements

can be considered as indicators for loss of consciousness, loss of cerebral cortex function, and spinal cord death (Jacobs et al., 2021). Although KED was as efficacious as the other three methods for kill success (57/59), use of the KED for broiler breeder euthanasia resulted in the longest duration of reflexes and the highest prevalence of broken skin and blood loss. These results were similar to previous work where reflexes and musculoskeletal movement lasted longer for broilers euthanized by KED than by CD (Bandara et al., 2019a; Jacobs et al., 2019; Baker-Cook et al., 2021). When KED was compared to CD, euthanasia of broilers using KED led to a greater vertebra fracture severity and a smaller separation distance between vertebrae which can be indicative of the vertebrae being forced apart (fractured) instead of mechanically stretched apart, which occurs during CD (Baker-Cook et al., 2021). This difference in the mechanism of spinal cord stretching/separation may have led to the observed differences in durations of reflexes. Although the absence of a nictitating membrane reflex is indicative of loss of consciousness, the presence of this reflex does not necessarily indicate consciousness since this is an autonomic reflex (Jacobs et al., 2021). The state of consciousness of birds euthanized by KED following euthanasia was not determined in our observations. Use of electroencephalogram recordings to assess level of unconsciousness during this immediate post-euthanasia time is further complicated due to the elevated electrical activity generated by the concurrent musculoskeletal clonic/tonic movements.

Captive bolt guns are commonly used to euthanize poultry and stun livestock prior to slaughter. TED was found to consistently cause near immediate insensibility (7 s), similar to previous research evaluating the effects of a percussion captive bolt (loss of visual evoked response at 24 s) (Gregory and Wotton, 1990). To result in immediate insensibility, it is necessary to fire the TED directly above the cranium covering the brain cerebrum. Lack of kill success using captive bolt has previously been hypothesized to be due to improper contact placement during euthanasia (Erasmus et al., 2010; Boyal et al., 2020). In a previous study, the effectiveness of 3 captive bolt guns (Cash Poultry Killer, TED, and Zephyr EXL) in causing irrecoverable unconsciousness was assessed on slaughter weight turkeys by evaluating behavioral and electroencephalographic indicators (Gibson et al., 2018). The results indicated that each of the evaluated captive bolt guns were effective for resulting in immediate unconsciousness/insensibility. Unsuccessful stuns were due to improper positioning of the captive bolt on the head, highlighting the importance of consistency (Gibson et al., 2018). Loss of consciousness and brain stem death was indicated by the absence or loss of the induced nictitating membrane reflex (Erasmus et al., 2010; Sandercock et al., 2014; Martin et al., 2016; Jacobs et al., 2021). Body movement is an expected result following severe brain trauma (TED) or severing the spinal cord (KED). The cessation of body movement is an indicator of spinal cord death (Dawson et al., 2007). The high frequency of broken skin

and blood loss were similar to observations seen when TED was used on turkeys and are inherent to the function of the TED (Jacobs et al., 2021). Although TED is a nonpenetrative captive bolt device, the skin is sufficiently damaged for blood loss to occur.

Although CO₂ euthanasia has typically been used for mass depopulation, it can also be used on individual poultry. The use of CO₂ at varying concentrations and combinations for poultry euthanasia has been frequently evaluated in the literature (Gerritzen et al., 1997; Kingston, et al., 2005; Coenen et al., 2007; Turner, et al., 2012; Baker et al., 2020). Throughout this experiment broiler breeders were found to display mouth gaping and head shaking during the euthanasia process, prior to loss of head posture followed by convulsions. However, due to the enclosure of the bird head within the CO₂ euthanasia device, assessment of physiological responses did not begin until the completion of the total euthanasia duration of 4 min. At this time, physiological responses that were observed during euthanasia had ceased. The time length that physiological responses persisted during the CO₂ euthanasia process are unknown. Because physiological observations were not initiated until the end of the euthanasia method, there were no or minimal eye nictitating membrane, reflexive mouth gaping, or physical movements. However, the time to cessation of heartbeat did not differ between CO₂, TED, and KED when assessment began at the end of the euthanasia method. If assessment had been initiated at the beginning of the CO₂ euthanasia method, this would have artificially lengthened the time to cessation of heartbeat. When considering which method is most appropriate for use in a particular situation, the length of time needed to apply CO₂ euthanasia should be considered.

The application of electrical euthanasia utilized transfer of an electrical current through the brain and heart of the broiler breeder. This resulted in unconsciousness and cardiac fibrillation with the complete cessation of convulsions following application (Boyal et al., 2020). In a previous study, researchers concluded that the use of intravenous sodium pentobarbital is the “gold standard” euthanasia method in turkeys causing immediate insensibility followed by death within 60 s (Hernandez et al., 2019a). Use of electrical euthanasia required application for only 15 s and was followed by a complete lack of body movement or heartbeat which indicates that electrical euthanasia is acceptable and potentially optimal for poultry application. However, compared to the other methods evaluated, electrical euthanasia had the disadvantage of not being completely portable. The use of electrical euthanasia also requires an adequate understanding of electrical wiring, current flow, and safe assembly (incorporation of a ground fault circuit interrupter GFCI) for application on poultry. There are 2 safety precautions for the operator as part of the device used in this study, a “current on” indicator light and GFCI outlet, allowing the operator to avoid contact with electrical current during application of electrical euthanasia (Boyal et al., 2020). Overall, with reflexes

and movements absent postapplication and no blood loss or broken skin, electrical euthanasia was demonstrated to be an effective euthanasia method.

The differences observed between broiler breeder males and females for the eye nictitating membrane, reflexive mouth gaping, and body movement durations indicate that although broiler breeder males and female are of similar body weight, they had different responses following application of KED or TED euthanasia methods. The longer duration of these reflexes for males compared to females may indicate extended times to attain total-brain death for males when these methods were used and then need for extended posteuthanasia observation time prior to carcass disposal.

Heartbeat Cessation

While electrical euthanasia led to heart fibrillation, the heart continued to beat following KED, TED, and CO₂ euthanasia for 427 to 659 s. Time to heartbeat cessation observed for broiler breeders was similar to a previous study evaluating CO₂ euthanasia in ducks and turkeys. When ducks and turkeys were euthanized with 40% CO₂, heartbeat cessation occurred at 588 s for ducks and 528 s for turkeys (Gerritzen et al., 2006a). However, in other studies, the time to heartbeat cessation was shorter. When multistage CO₂ stunning was evaluated, cessation of heartbeat was observed between 244 and 354 s (Gerritzen et al., 2013). Heartbeat cessation occurred at 209 s posteuthanasia following application of the TED to laying hens (Bandara et al., 2019b). In a study using broilers (5–11-wk-old), heartbeat cessation occurred at 274 s with foam euthanasia and 538 s and when birds were tented and exposed to CO₂ (Benson et al., 2007). These differences in time to heartbeat cessation indicate that bird age, bird species, and euthanasia method influence the time required to achieve death as determined confirmed by heartbeat.

Blood Plasma Corticosterone

The plasma corticosterone levels observed did not differ between pre- and post-euthanasia or between the applied euthanasia methods. In other studies, corticosterone following CO₂ euthanasia has been shown to be higher than pretreatment control values. Following mass euthanasia of broilers using tenting or foaming with and without CO₂, corticosterone levels increased for both treatments following euthanasia (Benson et al., 2007). A similar effect was observed in male layer chicks, where corticosterone levels were lower following euthanasia by gas inhalation (CO₂, N₂, or low atmospheric pressure) compared to decapitation without prior gas exposure (Gurung et al., 2018). The lack of differences in observed blood plasma corticosterone indicate that the 4 euthanasia methods are equivalent based on this stressor response indicator when birds are restrained in a MBEA and the amplitude of posteuthanasia convulsions are restricted. It was observed that males had higher levels

of blood plasma corticosterone than females prior to euthanasia and following TED or electrical euthanasia. This difference between male and female birds has been observed previously in mature Leghorns and market age turkeys with males having higher levels of corticosterone compared to females at the same age (Schmelting and Nockels, 1978; Jacobs et al., 2021). Elevated levels of corticosterone in males compared to females may be due to physiological and hormonal differences found in mature poultry (Schmelting and Nockels, 1978).

Overall, mechanical cervical dislocation using KED, captive bolt euthanasia using TED, individual bird CO₂ euthanasia, and electrical euthanasia were all appropriate methods for the euthanasia of mature broiler breeder chickens. If the operator's ability to apply manual CD efficiently and effectively for euthanasia is uncertain, alternative euthanasia methods evaluated would be preferable.

AUTHOR CONTRIBUTION STATEMENT

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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DISCLOSURES

The authors declare no conflicts of interest.

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