ANIMAL WELL-BEING AND BEHAVIOR

Animal welfare assessment of on-farm euthanasia methods for individual, heavy turkeys

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ABSTRACT On-farm euthanasia of poultry, including turkeys, may not be possible for most people as birds gain weight; thus alternative mechanical methods have been developed. Our objective was to compare mechanical cervical dislocation with the Koechner Euthanizing Device (**KED**), captive bolt euthanasia with the Turkey Euthanasia Device (**TED**), head-only CO_2 euthanasia (**CO_2**), and electric euthanasia as potential humane methods for euthanizing individual, heavy turkeys. We assessed their impact on loss of brain stem reflexes, acute distress (corticosterone, **CORT**), kill success, torn skin, and blood loss. Turkeys (n = 174) were euthanized on 3 sampling days, while birds were restrained using a mobile bird euthanasia apparatus. Brain stem reflexes recorded were the cessation and return of induced nictitating membrane reflex (loss of consciousness and brain stem dysfunction), mouth gaping reflex (brain stem dysfunction), and musculoskeletal movements (spinal cord dysfunction). Overall, KED resulted in more frequent (at 4 min: KED 7 of 14; electric 0 of 13; TED 0 of 11; CO_2 2 of 14 birds on day 1) and longer durations of the induced nictitating reflex compared to the other methods (means of day 2 and 3: KED 233; electric 15; TED 15; CO_2 15 s). The mouth gaping reflex endured the longest after KED euthanasia (means of day 2 and 3: KED 197; electric 15; TED 51; CO_2 15 s). Musculoskeletal movements endured longest after KED euthanasia (means of day 2 and 3: KED 235; electric 15; TED 219; CO_2 15 s). Returning reflexes were more frequent after KED and TED compared to CO_2 and electric euthanasia, where it was absent. CO_2, electric, and TED euthanasia showed comparable kill success (success: CO_2 42 out of 43; electric 44 of 45; TED 42 of 44), with KED resulting in most unsuccessful kills (unsuccessful: 8 out of 42). CORT responses were inconsistent. Torn skin and blood loss occurred more frequently after KED and TED compared to CO_2 and electric applications. Therefore, we conclude that, based on a comparison of these 4 methods, the most discernibly humane was electric euthanasia, which consistently resulted in quick loss of consciousness within 15 s, no returning reflexes, and no torn skin or blood loss.

Key words: Turkey, mechanical cervical dislocation, captive bolt, electrical euthanasia, animal welfare

2021 Poultry Science 100:100812
https://doi.org/10.1016/j.psj.2020.11.001

INTRODUCTION

On-farm euthanasia is needed when poultry, including broiler chickens and turkeys, are unhealthy, immobile, or otherwise incapacitated. The American Veterinary Medical Association recently published recommendations for euthanasia for poultry, including "gas inhalation, manually applied blunt force trauma, cervical dislocation, decapitation, electrocution, gunshot, captive bolt, and injectable agents" (Leary et al., 2020). A survey in Belgium showed that 2 out of 4 of turkey producers and 92% of veterinarians always choose manual cervical dislocation for individual turkey euthanasia without considering bird weight (Watteyn et al., 2020). Alternative methods of euthanasia need to be evaluated, because manual cervical dislocation requires physical strength from the operator and is susceptible to fatigue (Martin, 2015; OIE, 2017). Furthermore, consistent kill success can depend on bird weight, size, and age (Erasmus et al., 2010a; Martin et al., 2018), especially for turkeys, with turkey toms weighing between 7 and 20 kg at the

Received May 11, 2020.

Accepted November 2, 2020.

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end of production (Poultry Hub, 2020). As they age, their large body weight can limit the operator's ability to even lift the bird. In addition, legislative restrictions in Europe disallow the use of manual cervical dislocation for birds over 3 kg (European Union, 2009). Some industry stakeholders have expressed their willingness to try alternative methods. In the Belgian survey, 69% of veterinarians and 2 out of 4 turkey producers indicated their willingness to use euthanasia methods other than manual cervical dislocation (Watteyn et al., 2020).

A number of mechanical devices have been developed to overcome problems associated with manual cervical dislocation, such as the Koechner Euthanizing Device (**KED**, Clear View Enterprises LLC, Springdale, AR). A few studies have shown that mechanical cervical dislocation, either by crushing or stretching the neck and spinal cord, may not lead to immediate brain death in turkeys or chickens (Gregory and Wotton, 1990; Erasmus et al., 2010a,b; Baker, 2019; Hernandez et al., 2019; Jacobs et al., 2019). Furthermore, euthanasia attempts using the KED are not always successful, with a success rate of 54 and 88% in 8 and 18-week-old turkeys "in a light anesthetic state" (isoelectric electroencephalogram [**EEG**] at 5 min post application, Hernandez et al., 2019). Regardless of the variable success recently reported, mechanical cervical dislocation is recommended for euthanizing poultry (Leary et al., 2020).

Captive bolt euthanasia, aimed at causing extensive brain damage, hemorrhage, and rapid brain stem death, has successfully been applied for poultry euthanasia. A number of penetrative and non-penetrative captive bolt devices are commercially available, such as the Turkey Euthanasia Device (**TED**, Bock Industries, Philipsburg, PA). Relatively high success single application rates were reported for captive bolt euthanasia in turkeys, between 90 and 100% (Hulet et al., 2013; Gibson et al., 2018; Woolcott et al., 2018a).

Carbon dioxide (**CO_2**) inhalation is used in poultry processing plants as a stunning method prior to exsanguination or is used for whole-house gas depopulation during disease outbreaks (Gerritzen et al., 2006, 2007). With prolonged exposure, CO_2-induced hypoxia results in brain death. Individual 6 to 7-week-old turkeys lose consciousness at a level < 25% CO_2 (Gerritzen et al., 2006). Head-only CO_2 euthanasia could be a viable euthanasia method for individual birds on farm; however, data are needed to support this method.

Electrocution is recommended as a method of euthanasia for poultry (Leary et al., 2020), but on-farm tools to apply this method are limited in availability. Furthermore, connecting to a power source on-farm may be difficult. Electrocution results in cardiac fibrillation, cerebral hypoxia, and brain death (reviewed by Leary et al., 2020). Research on electrocution for on-farm euthanasia of poultry is lacking and the method requires adequate training to mitigate concerns for operator safety (Boyal et al., 2020).

When evaluating on-farm euthanasia for individual, heavy turkeys, we considered 4 operator-related criteria and 4 animal welfare-related criteria. From an operator point of view, euthanasia methods should 1) be manageable and safe to perform, 2) allow for mobility (moving within the farm or animal housing facility), 3) be successful when performed by a single operator, and 4) ensure success with a single application. Mechanical cervical dislocation, captive bolt euthanasia, head-only CO_2 euthanasia, and electrocution were all deemed to meet the 4 criteria if operators are properly trained (Boyal et al., 2020). From an animal welfare point of view, the methods should result in quick loss of consciousness (inability to perceive pain in the cerebral cortex), limited acute distress (elevated blood corticosterone [**CORT**]), and should be consistently successful after a single attempt. These animal welfare related factors were evaluated in the current study. Our objective was to compare 4 euthanasia methods for individual, heavy turkeys as potential humane methods for euthanizing individual, heavy turkeys in the context of culling, not depopulation. We evaluated this by assessing their impact on loss of reflexes, acute distress, kill success, torn skin, and blood loss. We focused on methods of euthanasia aimed at killing individual birds. We hypothesized that all methods of euthanasia would be appropriate for on-farm use. We expected that electric euthanasia would result in the most discernibly humane death, followed by TED, CO_2, and KED, respectively.

MATERIALS AND METHODS

Birds and Procedures

This experiment was approved by the Institutional Animal Care and Use Committee (**IACUC**) of Virginia Tech (IACUC protocol 18-124) and followed standard operating procedure #10 "Euthanasia Methods Approved for Poultry" approved by the IACUC at the U.S. National Poultry Research Center. On 3 sampling days, we euthanized batches of healthy, full-fed 21-week market turkeys or breeder turkeys (67 or 38 wk of age) at flock termination. The timing of our sampling (at flock termination) was chosen for 2 reasons: 1) we aimed to euthanize individual, heavy birds, and birds are heaviest at flock termination, and 2) the use of clinically healthy birds avoided potential confounding associated with underlying conditions. These birds came from flocks unrelated to the current study; thus, housing and husbandry were not controlled under our protocols. Four euthanasia methods were applied on each sampling day, with methods alternating to avoid order effects. An overview of bird details per sampling day is shown in Table 1. All details on restraint and euthanasia methods are described by Boyal et al. (2020).

Birds were caught and crated (sampling day 1 and 2; Table 1) or herded into a holding area of the pen and caught (day 3; Table 1). After catching, birds were weighed, manually inverted, and placed into a cone on day 1 and 2. On day 3, birds were caught, blood was collected, birds were weighed, and then placed in the cone. Immediately after placement in the cone, the euthanasia method was applied (Table 2), reflexes were assessed, and after death was

Table 1. Bird strain, age, bird numbers, sex distribution, and average live weight (BW ± SD) per sampling day.

				Bird n (mean BW ± SD; kg)		
Sampling date	Sampling day	Strain	Age (week)	Hens	Toms	Total
03 Oct 2018	1	Beltsville Small White	67	40 (3.51 ± 0.41)	16 (8.22 ± 0.48)	56
15 Apr 2019	2	Beltsville Small White	38	N/A	64 (6.62 ± 0.84)	64
10 Dec 2019	3	Broad-Breasted White	21	31 (12.80 ± 1.33)	23 (18.83 ± 1.23)	54
						174

Abbreviation: N/A, not applicable.

confirmed (cessation of musculoskeletal movements) postmortem blood was drawn.

Bird Restraint

For all but the electric euthanasia method on the Broad-Breasted White turkeys, birds were restrained in a cone, which allowed each method to be performed by a single, experienced operator and restricted the clonic/tonic convulsions that could occur after euthanasia. We constructed a mobile bird euthanasia apparatus (Boyal et al., 2020), consisting of a plastic traffic cone attached to a gas cylinder cart and mounted at a 45° angle, allowing a bird to be inserted into the cone with the head exposed for each euthanasia method. For the Broad-Breasted White turkeys (Table 1), a large metal bleeding cone (commonly used for processing) was required for restraint.

Euthanasia Methods

On sampling day 1, all euthanasia applications were performed by a single, trained operator (A) with 20 yr of experience in euthanizing poultry. This operator was trained in all the tested methods. On sampling day 2, two other trained operators (B and C) performed all CO_2 euthanasia, and operator A conducted the other methods. On sampling day 3, all but 1 bird were euthanized by operator B with 1 yr of experience with all methods. The one exception was euthanized by operator A.

Captive Bolt (TED) For captive bolt euthanasia, we used the non-penetrative TED (Bock Industries), which is a cordless, propane-powered tool modified by the seller from a commercial nail gun (Figure 1). Application was carried out according to manufacturer guidelines. The TED was cleaned with an air spray and the bolt lubricated between uses to ensure proper force and discharge consistency. A second TED was prepared on each sampling day as backup. After bird placement in the mobile bird euthanasia apparatus, the TED was placed at the crest of the head on an imaginary X between the back of the eyes and the ears, and the caruncle pulled caudally. The TED was either gently placed on the skin, or kept at approximately 1 cm distance from the head without any added adapter. After positioning, the trigger was pressed to apply a localized, non-penetrative blow to the head.

KED KED (KED-C and KED-T, Clear View Enterprises LLC) is a blunt plier-type device used for mechanical cervical dislocation (Figure 2). Application of KED-C (Beltsville Small White) and KED-T (Broad-Breasted White) included placement of the blades directly behind the base of the skull, with the twin blades ventral and the single blade dorsal, perpendicular to the head, neck, and median plane. Thereafter, the KED was gently closed until the blades touched the bird, which was followed by swift closing to separate the atlas neck vertebra from the skull.

CO_2 Stun-to-Kill (CO_2) A portable CO_2 inhalation euthanasia device was developed (Boyal et al., 2020; Figure 3) using a face mask to perform head-only gas stun-to-kill with a maximum concentration of up to 70% CO_2 at 1 min post-application, followed by a concentration increase to over 70% for the next 3 min. In addition, a similar, commercially available head-only gas device was used (CAS-3, QC Supply, Schuyler, NE; Donald James, Prestage Farms Inc., Clinton, NC). The CO_2 concentrations were measured with a gas analyzer (F-920 Check It! Gas Analyzer, Felix Instruments, Camas, WA). The CO_2 exposure resulted in gas asphyxiation and induced brain hypoxia.

Vent-to-Mouth Electrocution (Electric) The fourth euthanasia method was vent-to-mouth electrocution. The device was constructed with parts from a local hardware store, including an electrical box with a waterproof cover, a ground fault outlet, and a toggle switch (Boyal et al., 2020; Figure 4). The device had 2

Table 2. Bird numbers per euthanasia method, per sampling date.

	Method								
	CO_2		Electric		KED		TED		
Sampling day	Hens	Toms	Hens	Toms	Hens	Toms	Hens	Toms	Total n
1	10	4	10	4	10	4	10	4	56
2		16		16		16		17	64
3	7	6	7	6	7	5	7	6	54
Total n	17	26	17	26	17	25	17	27	

Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

Figure 1. The Turkey Euthanasia Device (TED) is a nonpenetrative captive bolt tool for single-bird euthanasia.

safety features: an indicator light on a grounding adapter, which allowed the operator to visually determine whether electricity was on, and the ground fault outlet to protect the operator from electrical shock. Attached to the top of the device was a weatherproof cover to prevent water from entering the electrical box when the device was not in use. An electrical cord was wired to the electrical box through a conduit fitting, and plugged into an electrical outlet. A split electrical cord was connected to 2 battery clips and plugged into the outlet on the electrical box.

Birds were euthanized by placing a clip (Figure 4D) on the vent first and the mandible second. This order was deemed more humane based on birds' discomfort from the clip on the jaw (D. V. Bourassa, personal observation). The operator then immediately turned on the electricity (120 V AC, 60 Hz) for 15 s (Figure 4C). After that time duration, the electrical current was turned off and the electrode clips removed.

Figure 2. The Koechner Euthanizing Device (KED) used for mechanical cervical dislocation.

Measurements

Brain Stem Death Brain stem death and loss of consciousness were assessed by observing the loss or absence of induced nictitating membrane reflex (Erasmus et al., 2010a; Sandercock et al., 2014; Martin et al., 2016), the mouth gaping reflex (Erasmus et al., 2010a) suggesting loss of cerebral cortex control, and cessation of musculoskeletal movements (Dawson et al., 2007), suggesting spinal cord dysfunction. Assessments were similar to the tests described in our previous work on broiler chicken euthanasia (Jacobs et al., 2019). On the first sampling day (Tables 1 and 2), brain stem reflex measurements were taken only at 4 min after application. On the second and third sampling days (Table 1), measurements were taken every 15 s (presence or absence), starting 15 s after application of the euthanasia method. The time was recorded from the application of the euthanasia method until 1 min after cessation of musculoskeletal movements. The absence of an induced nictitating membrane reflex was considered a primary indicator of lack of brain stem function and loss of consciousness (Erasmus et al., 2010c). This reflex was assessed by touching the medial canthus of 1 eye after the euthanasia method was applied, with birds being considered to maintain brain stem functionality if the third eyelid was observed to move over the cornea. We considered the mouth gaping reflex to be a brain stem reflex mediated by the cranial nerves (Schmidt and Wild, 2014), which was evaluated by observing the opening and closing of the beak. The third, most conservative indicator of brain, brain stem, and spinal cord death was the cessation of all musculoskeletal movement (including vent, legs, and tail). We interpreted loss of musculoskeletal movement as confirmation of brain, brain stem, and spinal cord death. Presence of these movements were not interpreted as consciousness. Latency to the first loss of reflex was recorded, that is the first time point when no reflex was observed, after the last reflex was observed (s), plus the return of reflexes or musculoskeletal movements (yes or no). For CO_2 euthanasia, the first reflex assessment took place 4 min + 15 s after the start of the euthanasia application, which was directly after the end of CO_2 exposure. For electric euthanasia, the first reflex assessment took place 15 s after the start of the euthanasia application, as the birds were exposed to the electrical current for 15 s.

Acute Stress Response Blood was collected to assay plasma CORT. On sampling day 1, postmortem blood was collected through a cardiac puncture after the bird was confirmed dead. The abdominal cavity was opened and a needle was inserted in the heart ventricle and 2 to 3 mL was collected in a lithium-heparin flushed tube. On day 3, blood was collected from the brachial vein prior to euthanasia to find a possible explanation for individual variabilty in CORT responses after euthanasia. We used a 21G needle on a lithium-heparin flushed 10-mL syringe. Handling duration was recorded for the antemortem blood collection, starting when the bird was caught in the pen until the needle was removed

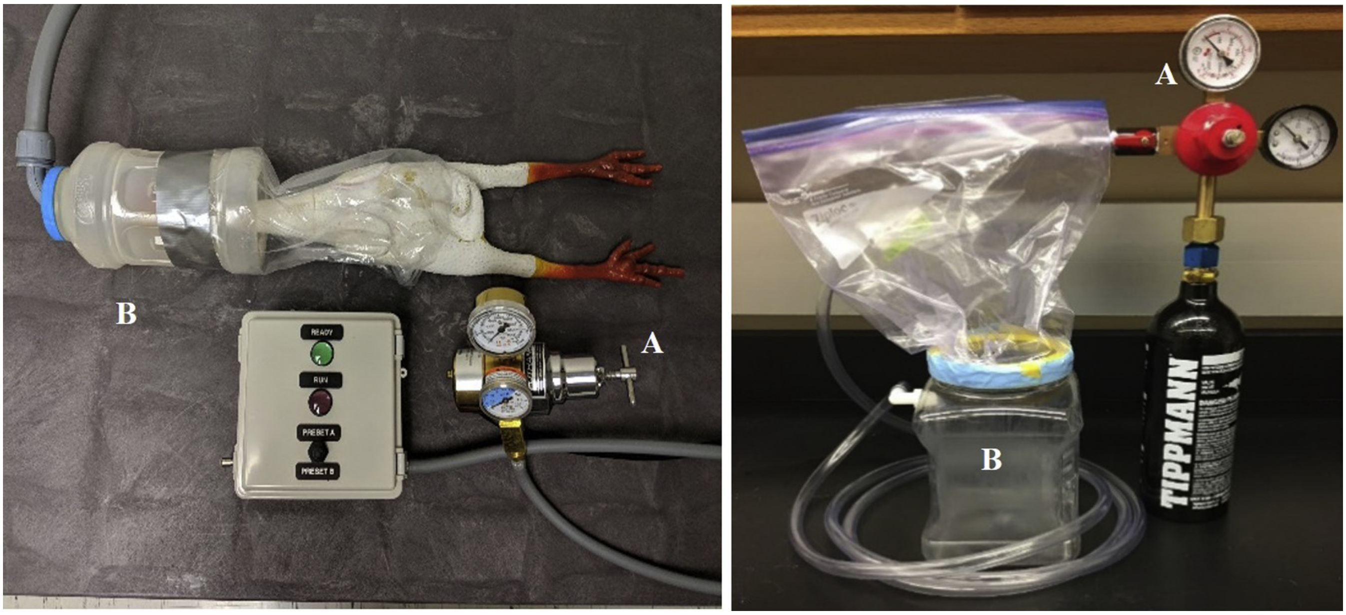

Figure 3. The CO_2 euthanasia system included a 0.6-L CO_2 tank, regulator (A), hose, plastic chamber (B), and accessories, which allowed head-only, single bird euthanasia.

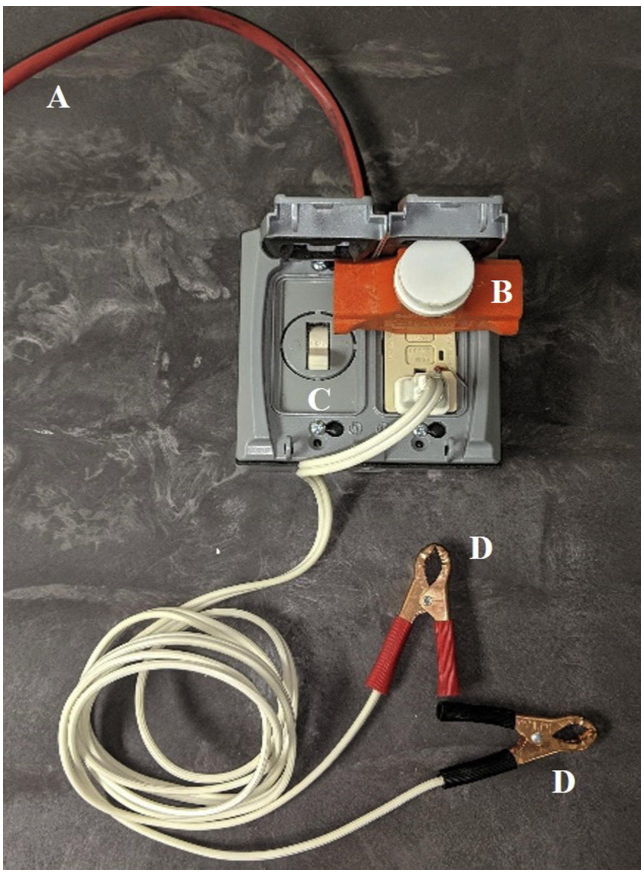

Figure 4. Euthanasia device for vent-to-mouth electrocution. The device consisted of readily available parts from a local hardware store. A indicates the cord that was plugged in an outlet. B represents the safety light, which lit up when electricity was turned on. C is the toggle switch turning electricity on or off. D refers to the clips placed on the vent and into the mouth, over the mandible.

from the vein. Mean handling duration was 82.4 s (min 50 s, max 153 s; n = 53). Postmortem blood collection on day 3 was from a neck cut holding the collection tube directly under the cut. Blood samples were placed on crushed ice and centrifuged at 3,000 rpm for 15 min to separate plasma from whole blood. Plasma was pipetted out and placed in another tube, which was stored on ice during the day, and stored at −20°C until assayed. Plasma CORT was assayed using an ELISA kit, following the manufacturer's protocol (item no. 501320, Cayman Chemical, Ann Arbor, MI). Samples were assayed in triplicate and 3 or 2 out of 3 replicates were selected to achieve an intra-assay CV below 15% (intra-assay CV average was 7.8%).

Kill Success and Torn Skin On sampling day 1, kill success was recorded (yes or no) at 4 min post application. On the other sampling days, kill success was defined as a single euthanasia attempt needed to achieve no signs of recovery, which was determined by the absence of (returning) respiration, regardless of duration (Martin et al., 2016). The presence of torn skin and bleeding from the head, neck, or mouth were noted postmortem (present or absent) as indicators of physical damage following euthanasia.

Data Analysis

Dependent variables included latency to loss of brain stem death reflex (s; nictitating membrane reflex, mouth gaping reflex), spinal cord death (musculoskeletal movements), ante and postmortem CORT concentration (ng/mL), return of reflexes, kill success, and torn skin (all yes or no; binominal). All dependent variables were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using

Table 3. Frequency of observations of reflexes and musculoskeletal movements on sampling day 1, scored at 4-minute post-euthanasia application for CO_2 euthanasia, vent-to-mouth electrocution (electric), KED, and TED.

	At 4-minute post-euthanasia				
	Nictitating membrane reflex		Musculoskeletal movement		
Euthanasia method	Present	Absent	Present	Absent	Total n
CO_2	$2^{a,b}$	12	$2^{a,b}$	12	14
Electric	0^{b}	13[1]	0^{b}	14	14
KED	7^{a}	7	7^{a}	7	14
TED	0^{b}	11[1]	$2^{a,b}$	12	14

[a,b] Values within a column lacking a common superscript differ ($P < 0.05$).

Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

[1]Missing data for this measure.

nonparametric methods to test the effect of the euthanasia method, as data residuals were not normally distributed. We used the Wilcoxon rank sum test for multiple comparisons, evaluating the outcomes of the Kruskal-Wallis test. If the effect of the euthanasia method was significant at $P < 0.05$, post hoc pairwise comparisons were performed with a Benjamini-Hochberg correction to control for the expected proportion of false discoveries in R (R Core Team, Vienna, Austria, 2013). The effect of bird sex on frequencies (sampling day 1 and 3) and durations (day 3) was tested using nonparametric methods (Kruskall-Wallis chi-square). The effect of body weight on outcome variables was tested using Pearson correlation coefficients. Results are presented as raw means ± SE, frequencies, or proportions for each sampling day (1, 2, 3).

RESULTS

Brain Stem Death Reflexes

On sampling day 1 (Table 3) when reflexes were scored 4 min post-application, differences in the presence of reflexes and movements were found for both the nictitating membrane reflex ($P = 0.003$) and musculoskeletal movements ($P = 0.012$). The nictitating membrane reflex was more commonly observed after KED applications compared to the electric applications (pairwise $P = 0.025$). Similarly, the nictitating membrane reflex was more common after KED compared to TED euthanasia (pairwise $P = 0.033$). In addition, musculoskeletal movements were more commonly observed after KED compared to electric euthanasia (pairwise $P = 0.018$). No other pairwise differences were observed ($P \geq 0.130$).

On sampling day 2 and 3, latencies to loss of reflexes and musculoskeletal movements (Figure 5) differed between the 4 euthanasia methods ($P < 0.0001$ for loss of nictitating membrane reflex, mouth gaping reflex, and musculoskeletal movements on both sampling days).

CO_2 and electric methods consistently showed shorter latencies to loss of nictitating membrane reflexes, mouth gaping reflexes, and musculoskeletal movements compared to KED euthanasia (all pairwise $P < 0.001$ for sampling day 2 and 3). CO_2 and electric methods showed shorter latencies to loss of musculoskeletal movements compared to TED (all pairwise $P < 0.0001$ for sampling day 2 and 3). Nictitating membrane and mouth gaping reflex durations did not differ between CO_2, electric, or TED euthanasia ($P \geq 0.1$ on sampling day 2, and $P \geq 0.35$ on sampling day 3). Musculoskeletal movements did not differ between CO_2 and electric euthanasia. When comparing TED to KED euthanasia, TED applications resulted in shorter latencies to loss of nictitating membrane reflex (pairwise $P < 0.0001$ for both sampling days), and mouth gaping reflexes (pairwise $P < 0.001$ for both sampling days). No pairwise difference was found for latency to loss of musculoskeletal movements between TED and KED ($P = 0.21$ on sampling day 2 and $P = 0.12$ on sampling day 3). On sampling day 2, the latency to loss of musculoskeletal movements was scored during CO_2 exposure, and resulted in a mean latency of 81 s (n = 14, SD = 16 s).

The frequency reflexes that returned after euthanasia differed between euthanasia methods on sampling day 2

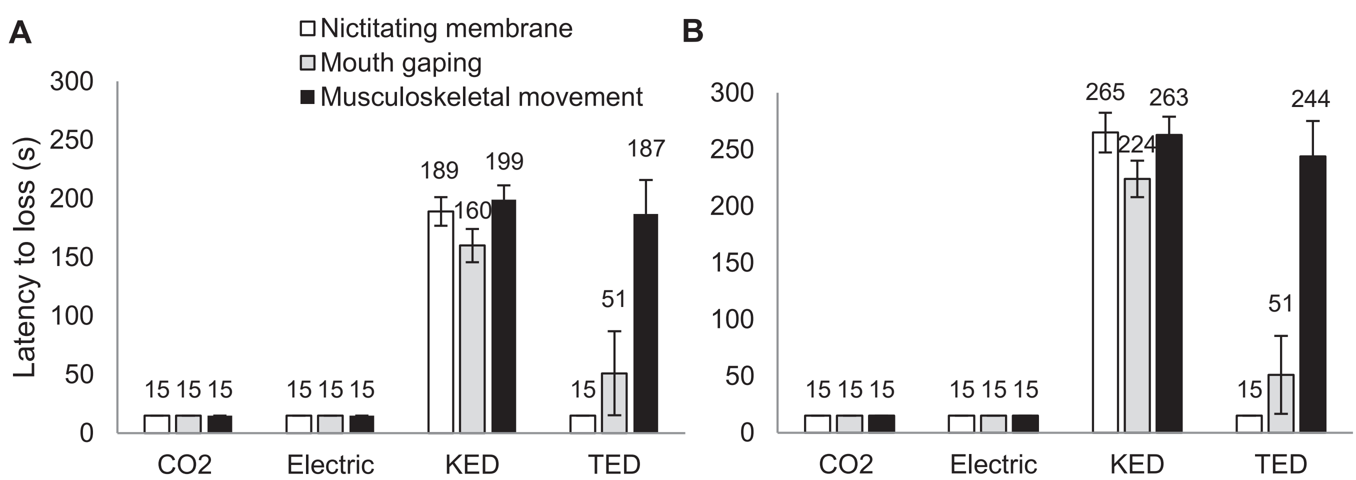

Figure 5. Means ± SEM for latency to loss of reflexes and loss of musculoskeletal movements (s) for the 4 euthanasia methods (CO_2, vent-to-mouth electrocution, mechanical cervical dislocation [KED], captive bolt [TED]) on sampling day 2 (A) and 3 (B). Means of 15 s reflect absence of reflexes at first assessment after euthanasia application (n = 12–17 per method per sampling day). Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

($P < 0.0001$), and tended to differ on sampling day 3 ($P = 0.053$), with more frequently returning reflexes after KED and TED euthanasia compared to CO_2 and electric euthanasia (Table 4).

Acute Stress Response

On day 3, CORT concentrations prior to restraint and euthanasia were assessed, and no differences between methods were found ($P = 0.545$), with mean concentrations (±SE) of 9.1 ± 0.8 ng/mL for electric, 11.2 ± 1.8 ng/mL for CO_2, 10.4 ± 2.9 ng/mL for TED, and 10.3 ± 1.2 ng/mL for KED.

Post-euthanasia plasma CORT concentrations differed between euthanasia methods on sampling day 1 ($P = 0.010$; Figure 6). Electric euthanasia resulted in lower CORT concentrations compared to CO_2, KED, and TED euthanasia (pairwise $P = 0.018$ for all comparisons on sampling day 1), with mean concentrations (±SEM) of 3.7 ± 2.5 ng/mL for electric, 6.9 ± 2.5 ng/mL for CO_2, 8.5 ± 4.4 ng/mL for TED, and 9.6 ± 3.7 ng/mL for KED. Other pairwise comparisons were not significant on sampling day 1 (pairwise $P = 1$). Post-euthanasia CORT concentrations did not differ between methods on sampling day 3 (post: $P = 0.979$; Figure 6), nor was there a difference in the CORT concentration change comparing pre-euthanasia and post-euthanasia concentrations ($P = 0.350$).

Kill Success and Torn Skin

Kill success differed between methods on sampling day 1, but not on day 2 or 3 ($P < 0.001$, $P = 0.422$, and $P = 0.785$, respectively; Figure 7). CO_2 (n = 13 of 14 successful kills), electric (n = 14 of 14), and TED (n = 14 of 14) euthanasia resulted in more successful kills compared to KED euthanasia (n = 7 of 14) on day 1 (pairwise $P < 0.025$ for all comparisons). Other methods did not significantly differ in kill success.

External damage (torn skin and external blood loss) differed between euthanasia methods ($P < 0.0001$ for both torn skin [day 1, 2, and 3] and blood loss [day 2 and 3]). CO_2 and electric euthanasia consistently resulted in no torn skin or external blood loss (Figure 8). CO_2 and electric euthanasia showed no torn skin compared to KED and TED euthanasia on all sampling days (pairwise $P \leq 0.022$ for all comparisons). KED and TED caused similar torn skin prevalences on day 1 and 3 (pairwise $P = 0.16$ and 0.15), but TED did result in more frequently torn skin on sampling day 2 (pairwise $P < 0.001$). Similarly, external blood loss was absent after CO_2 and electric euthanasia, compared to KED and TED euthanasia (pairwise $P \leq 0.022$ for all comparisons). KED and TED resulted in similar blood loss frequencies on day 3 (pairwise $P = 0.15$), but more frequent blood loss after KED than TED euthanasia on day 2 (pairwise $P < 0.0001$).

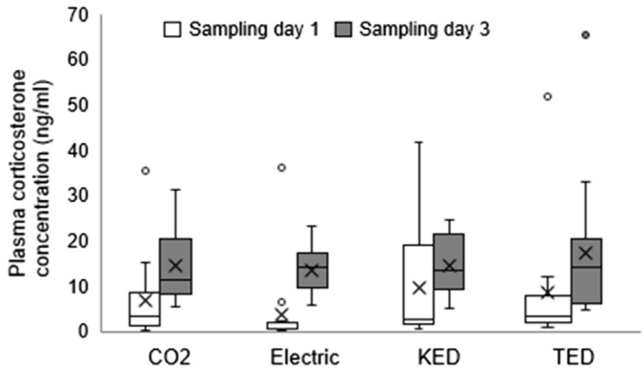

Figure 6. Sampling day 1 (white) and 3 (grey) post-euthanasia (CO_2, vent-to-mouth electrocution, mechanical cervical dislocation [KED], captive bolt [TED]) plasma corticosterone concentrations (ng/mL). The boxplot diagrams show medians (lines in the boxes), means (x), 25 and 75% quartiles (boxes), 10 and 90% ranges (whiskers), and outliers (dots) of corticosterone concentrations (n = 11–14 per method per sampling day). Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

Table 4. Frequency (n and %) of reflexes or musculoskeletal movements returning after a single euthanasia attempt on sampling day 2 and 3.

	Returning reflexes			
	Sampling day 2		Sampling day 3	
Euthanasia method[1]	n	%	n	%
CO_2	0^b	0	0^B	0
Electric	0^b	0	0^B	0
KED	12^a	75	3^A	25
TED	8^a	47	3^A	23

[a,b]Values within a column lacking a common superscript differ ($P < 0.05$).

[A,B]Values within a column lacking a common superscript differ ($P < 0.1$).

Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

[1]CO_2, vent-to-mouth electrocution, mechanical cervical dislocation (KED), captive bolt (TED).

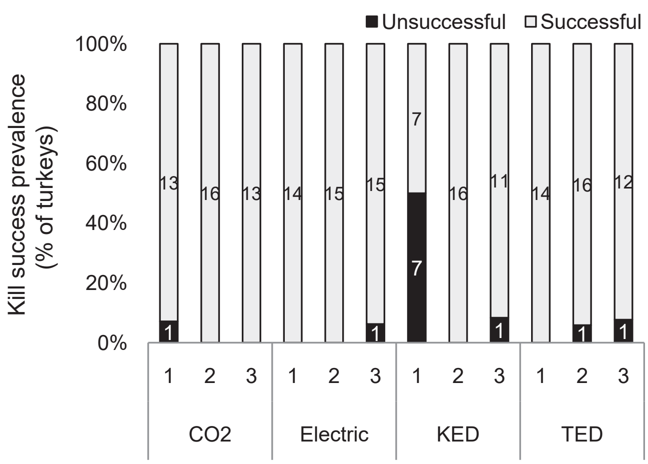

Figure 7. Prevalence of successful and unsuccessful kills per euthanasia method (CO_2, vent-to-mouth electrocution, mechanical cervical dislocation [KED], captive bolt [TED]) per sampling day (1, 2, and 3) (n = 12–17 per method per sampling day). Numbers within column represent absolute bird numbers. Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

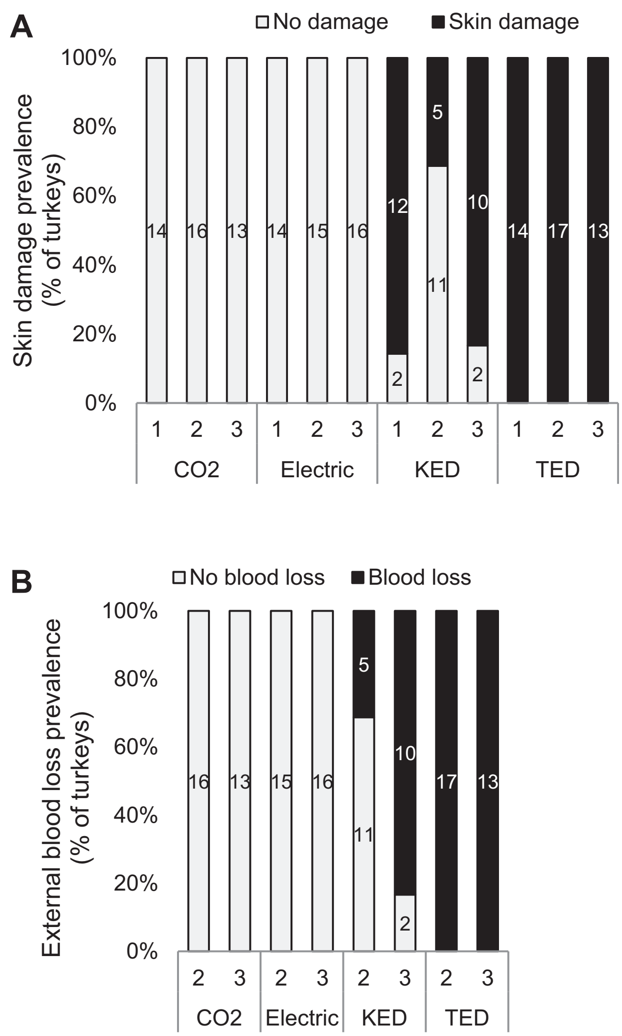

Figure 8. (A) Prevalence of skin damage (% of birds with or without damaged skin) and (B) prevalence of external blood loss after application of each euthanasia method (CO_2, vent-to-mouth electrocution, mechanical cervical dislocation [KED], captive bolt [TED]), on 3 or 2 sampling day. Numbers within columns represent absolute bird numbers. Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.

Bird Sex and Weight

Bird sex did not impact the presence of reflexes and movements (day 1), prevalence of returning reflexes, external blood loss, external damage, or kill success on sampling day 1 or 3 ($P > 0.385$). Latencies to loss of reflexes and movements were not affected by bird sex on day 3 ($P > 0.336$). Pre-euthanasia CORT concentrations did not differ between sexes on day 3 ($P = 0.193$). After euthanasia, CORT concentrations did not differ on day 1 ($P = 0.165$), but did differ between males and females on day 3 ($P = 0.024$). Males had higher mean circulating CORT concentrations (16.0 ± 1.3 ng/mL) than females (14.0 ± 2.1 ng/mL). Body weight was not correlated with the prevalence of reflexes and movements (day 1), or latencies to loss of reflexes or movements after euthanasia on day 2 or day 3 ($P \geq 0.35$). Prevalence of external blood loss, damage, or kill success was not correlated to bird weight ($P > 0.19$). A tendency for a weak negative correlation was found between post-euthanasia CORT and bird weight ($r = -0.238$; $P = 0.090$) on day 1, but not on day 3 ($P = 0.77$).

DISCUSSION

We aimed to identify differences between the 4 euthanasia methods for reflex durations, acute stress responses, kill success, torn skin, and blood loss, with the ultimate objective to compare euthanasia methods for individual turkeys that could be applicable on-farm. Euthanasia methods were evaluated on 3 sampling days, with the results generally consistent between sampling days.

Brain Cerebral Cortex, Brain Stem, and Spinal Cord Death and Reflexes

We evaluated the loss of consciousness (nictitating membrane reflex), loss of cerebral cortex control functions (mouth gaping), and spinal cord death (musculoskeletal movements). A limitation of the current study is the lack of EEG data to support the behavioral and physiological responses to the tested euthanasia methods. However, previous work has shown associations and validation between the tested reflexes and onset of brain stem death (Dawson et al., 2009; Sandercock et al., 2014; Cors et al., 2015; Hernandez et al., 2019). It is noteworthy though that not all results are consistent, possibly due to the use of anesthesia in some studies (Mcilhone et al., 2014; Sandercock et al., 2014; Hernandez et al., 2019). In addition, EEGs are not interpretable in the presence of musculoskeletal movements (clonic/tonic convulsions) and that was a reason for using anesthesia in the cited studies.

We observed considerable differences in brain stem reflexes when comparing the euthanasia methods on all 3 sampling days. KED euthanasia generally resulted in prolonged presence of reflexes and musculoskeletal movements compared to the other methods. Large differences were found when comparing short latencies after CO_2 and electric methods to the prolonged latencies after KED euthanasia on day 2 and 3. Reflex durations did not differ between CO_2, electric, or TED euthanasia, although durations of musculoskeletal movements were longer after TED euthanasia (Table 5). For CO_2 euthanasia, we were unable to evaluate the duration of reflexes and movement during actual CO_2 provision, as reflex assessments started 15 s after the 4-minute CO_2 exposure.

Dysfunction of the brain stem is indicated as the interruption of afferent and efferent neuron impulses through the brain stem to and from the cerebral cortex; therefore birds are impervious to pain and unconscious. The absence of a functional brain stem would result in no ventilatory reflexes and death would follow. Therefore, the absence of a nictitating membrane response can be used as a conservative indicator of unconsciousness

Table 5. Mean durations (s) of nictitating membrane and mouth gaping reflexes, and musculoskeletal movements from sampling day 2 and 3, compared to literature findings (means; s) where poultry (broiler chickens, laying hens, or turkeys) of varying ages were euthanized with the same or similar methods.

		Euthanasia method[12]			
Measurement (unit)	Source	CO_2	Electric	KED	TED
Nictitating membrane reflex (s)	Current study[13]	15	15	233	15
	Literature			68[5]	0[10]
				106[2]	0[9]
				130[4]	1.8[11]
					2[8]
Mouth gaping reflex (s)	Current study[13]	15	15	197	51
	Literature			89[6]	0[10]
				117[7]	28[9]
				134[4]	
				253[3]	
Musculoskeletal movements (s)	Current study[13]	15	15	235	219
	Literature	565–700[1]		114[2]	120[8]
				202[3]	173–188[9]
					200–204[10]

Blank cells indicate lack of data in the literature.
Abbreviations: KED, Koechner Euthanizing Device; TED, Turkey Euthanasia Device.
[1]39 to 44% CO_2—latency to motionless from start of CO_2 exposure (Gerritzen et al., 2004).
[2]Burdizzo (plier-like device)—turkeys (Erasmus et al., 2010a).
[3]KED—turkeys (Hernandez et al., 2019).
[4]KED—young turkeys (Woolcott et al., 2018b).
[5]KED—broiler chickens (Baker, 2019).
[6]KED—broiler chickens (Jacobs et al., 2019).
[7]KED—laying hens (Bandara et al., 2019).
[8]TED—turkeys and broiler chickens (Hulet et al., 2013).
[9]Zephyr (captive bolt) and TED—turkeys (Woolcott et al., 2018a).
[10]Zephyr—turkeys (Erasmus et al., 2010a).
[11]Zephyr—broiler chickens (Baker, 2019).
[12]CO_2, vent-to-mouth electrocution, mechanical cervical dislocation (KED), captive bolt (TED).
[13]Combined raw means (s) of sampling day 2 and 3.

(insensibility). However, the presence of a nictitating membrane response cannot be used as an indicator of consciousness; it merely indicates the brain stem is functional. In other words, loss of the reflex indicated loss of consciousness, but presence of the reflex does not indicate consciousness. Individual animals can be unconscious and therefore the cerebral cortex may be unable to perceive pain while the brain stem reflex for respiration is maintained.

With the loss of the nictitating membrane reflex as the primary indicator of insensibility or unconsciousness, our results indicate that on sampling day 2 and 3 all birds lost consciousness within 15 s after application of CO_2, electric, and TED euthanasia (Table 5). Nictitating membrane reflex durations after TED euthanasia were comparable to turkeys or broiler chickens euthanized with the same or a similar captive bolt device (Erasmus et al., 2010a; Hulet et al., 2013; Woolcott et al., 2018a; Baker, 2019) (Table 5).

KED nictitating and mouth gaping reflex durations were longer compared to electric, CO_2, and TED euthanasia methods. Duration of musculoskeletal movements after KED euthanasia was similar to TED, but prolonged compared to CO_2 and electric euthanasia. Nictitating membrane reflex durations in KED turkeys were longer than previously reported in turkeys or broiler chickens using the same or similar devices (Table 5) (Erasmus et al., 2010a; Woolcott et al., 2018b; Baker, 2019).

The KED mouth gaping duration fell within the range of previously reported means for turkeys and broiler chickens (Woolcott et al., 2018b; Hernandez et al., 2019; Jacobs et al., 2019). However, conscious mouth gaping is also considered an initial behavioral response to the detection of CO_2 in the air (Lambooij et al., 1999). In our study, CO_2 euthanasia was performed using a head-only device, making observations during provision of CO_2 difficult. Therefore, initial—conscious—gaping during CO_2 inhalation was not structurally recorded; thus aversion and potential breathlessness could not be determined. We observed presence of the mouth gaping reflex for prolonged periods of time after KED and TED euthanasia (KED 197 s; TED 51 s; Table 5). Mouth gaping after TED euthanasia was prolonged compared to the means reported in other studies with turkeys or broiler chickens, using a similar or the same device (Erasmus et al., 2010a; Hulet et al., 2013; Woolcott et al., 2018a).

Musculoskeletal movements were the last to disappear compared to nictitating membrane reflex or mouth gaping reflexes, showing that it is a conservative indicator of spinal cord death (Dawson et al., 2007; Jacobs et al., 2019). In line, Hernandez et al. (2019) reported a strong positive correlation ($r^2 = 0.64$) between cessation of body movements and isoelectric EEG in turkeys after manual cervical dislocation (but not after mechanical cervical dislocation), suggesting brain death. Similarly, loss of posture coincided with isoelectric brain activity in some but not all broilers after CO_2 euthanasia (Gerritzen et al., 2004). Brain stem death was induced within 15 s after CO_2 and electric euthanasia, but took longer for TED and KED euthanasia. The latter findings are similar to durations reported using the Zephyr device (Erasmus et al., 2010a), but longer compared to other studies (Hulet et al., 2013; Woolcott et al., 2018a,b) (Table 5). After CO_2 euthanasia, cessation of movement occurred before 15 s post-application. Data collection on sampling day 2 showed a mean latency to loss of movements of 81 s during CO_2 exposure. This duration is considerably shorter than that reported in broiler chickens (Gerritzen et al., 2004), which is due to the different methods of exposing birds, with direct head-only exposure for 4 min in our study compared to whole room exposure (nearly 90 m^3) for 25 min in their study.

Returning reflexes were more frequent after KED (25 and 75% of birds) and TED euthanasia (23 and 47%), compared to CO_2 and electric euthanasia (0%). Reflexes returned in similar rates compared to observations in 5- and 6-week old broiler chickens, where reflexes returned in 50 to 55% of broilers euthanized with KED (Jacobs et al., 2019), but more frequently compared to findings from Erasmus et al. (2 of 32 turkeys with Zephyr; 2010a). Returning reflexes could possibly indicate animals recuperating or recovering after the euthanasia attempt. Returning reflexes and movement are possibly due to limited, more localized trauma, and an insufficient loss of blood supply to the brain (shown in rats; Dimar et al., 1999), which is a potential concern from an animal welfare perspective.

Acute Stress Response—CORT

To our knowledge, this is the first study assessing CORT concentrations in relation to euthanasia methods in turkeys. In humans, excessive CO_2 in the bloodstream as a result of CO_2 inhalation resulted in activation of the hypothalamo-pituitary-adrenal axis, which is responsible for increased CORT mobilization (Kaye et al., 2004). Neonatal chicks showed nonsignificantly different serum CORT concentrations from 6.3 to 7.8 ng/mL after euthanasia with 75 or 90% CO_2, 100% N_2, or low atmospheric pressure 20% (Gurung et al., 2018). Those concentrations were significantly lower compared to decapitated controls with CORT levels of 12.0 ng/mL (Gurung et al., 2018). Their responses are somewhat comparable to median concentrations of 3 and 11 ng/mL in our study. CORT concentrations of 1.8 ng/mL from broiler chickens electrically stunned at a commercial plant (after inversion and shackling) were comparable (sampling day 1) or lower than (sampling day 3) the median CORT concentrations in our study (0.7 and 14 ng/mL) (Vizzier-Thaxton et al., 2010). It should be noted that circulating CORT concentrations have been associated with arousal caused by an experience rather than valence (positive or negative) of the experience (Buwalda et al., 2012). Yet, in experiments focusing on euthanasia, the valence of the experience is likely to be negative; therefore arousal in these cases is a response to a negative stressor. With CORT concentrations influenced by many factors, such as time of day, handling, restraint (Kannan and Mench, 1996; Bedanova et al., 2007), in addition to euthanasia itself, variation is demonstrated within and between studies. We found a difference in CORT responses between euthanasia methods on sampling day 1, but not on day 3. Three main factors could have contributed to the differences between sampling days. Birds were collected differently; they were crated on day 1, and penned outdoors on day 3. Birds were of a different strain and age at the time of euthanasia (Table 1). Furthermore, additional blood collection occurred prior to euthanasia on day 3, but not on day 1. The acute stress from restraint and blood collection before application of euthanasia methods could have been the reason for CORT concentrations to peak, resulting in no differences between euthanasia methods in the postmortem concentrations on day 3. Additional indicators of acute distress may therefore be more reliable than CORT when evaluating the impact of euthanasia.

Kill Success, Torn Skin, and Blood Loss

CO_2, electric, and TED euthanasia attempts were consistently successful with the exception of 4 of 122 turkeys (sum of all turkeys euthanized with those methods). However, the application time for CO_2 being 4 min plus the post application time of 4 min resulted in a total time of 8 min to determine death. In contrast, KED euthanasia attempts were less successful, with 8 of 42 kills not resulting in spinal cord death after a single attempt. Ineffective euthanasia attempts (death = isoelectric EEG at 5 min) with KED were previously reported for 16 of 22 turkeys aged 8 and 18 wk (Hernandez et al., 2019). In contrast, our broiler chicken study did show a 100% success rate after a single KED attempt (Jacobs et al., 2019). Unsuccessful kills are an animal welfare concern when birds begin reinitiating respiration and therefore potentially recuperate after the euthanasia attempt. Training (Erasmus et al., 2010a; Martin et al., 2018), operator fatigue, and incorrect placement of the device are contributing factors to kill success. In these experiments, 7 of the 8 failed kills for KED occurred on day 1 at 67 wk of age (Figure 7) indicating that training improved efficacy on sampling day 2 and 3 where only 1 of 17 KED euthanasia attempts was determined as a failed kill. Kill success was not affected by bird weight or sex in our study, contrary to previous findings (Erasmus et al., 2010a; Martin et al., 2018).

When skin was torn, external blood loss occurred. Torn skin and blood loss were most common after TED euthanasia (100%), followed by KED euthanasia (63%). External damage after TED euthanasia was more frequent compared to the prevalence reported in turkeys (92 of 117, 78% after TED and 93 of 119, 78% after Zephyr) (Woolcott et al., 2018a). Torn skin after KED euthanasia (27 of 43, 63% prevalence) was comparable to findings in broiler chickens (46 and 53% prevalence; Baker, 2019). No skin tears were observed after

CO_2 or electric euthanasia. Initial testing (D. V. Bourassa, personal observation) of the electric method did not result in electrical burns in beak and vent areas, and was therefore not assessed as a parameter following euthanasia. Torn skin could be painful if birds were conscious (however, the immediate presence of convulsions indicated that it is unlikely), and external blood loss can pose an additional biosecurity hazard during disposal (as well as spilled crop contents and feces) if birds are contaminated (Cors et al., 2015).

Similarly to EEG and behavioral data presented in another recent study (Hernandez et al., 2019), our data suggest that KED (mechanical cervical dislocation) is not the quickest euthanasia method to achieve insensibility and death for turkeys. We found consistently persisting reflexes, prolonged musculoskeletal movements, returning reflexes, less frequent kill success at 4 min (only on sampling day 1, indicating improved efficacy with successive operator training and experience), and more frequently torn skin and external blood loss. Electric euthanasia was deemed most favorable for heavy, individual turkeys because of the quick loss of consciousness and onset of death, evaluated by the quick loss of reflexes and movements, lowest acute stress response (CORT) on day 1, frequent kill success, and lack of damage and external blood loss. Thereafter, CO_2 showed promising results in terms of the early onset of insensibility and death, kill success, and external damage. In line with other research (Woolcott et al., 2018a), TED was also deemed an effective alternative for turkeys; however, returning reflexes could be a cause for concern for animal welfare, and frequently torn skin and blood loss could be an additional biosecurity concern.

To conclude, we compared 4 euthanasia methods for individual, heavy turkeys that were deemed to be practical and safe from an operator point of view. To evaluate the methods, we assessed their impact on loss of consciousness (indicated by cessation of the nictitating membrane reflex), onset of brain stem death (cessation of mouth gaping) and spinal cord death (cessation of musculoskeletal movements), the acute stress response, and external physical damage (broken skin). Bird weight or sex did not impact these responses. Further research on refinement of euthanasia methods would be valuable. More work on identifying the potential perception of pain and distress associated with different euthanasia methods is needed. Based on our results, the recommended euthanasia method for individual turkeys between 3.5 and 18.8 kg is vent-to-mouth electrocution (electric). This method consistently resulted in quick loss of consciousness after application (within 15 s), no returning reflexes, no musculoskeletal movements (convulsions), and no external physical damage. Efficiency for depopulation would need to be assessed in a future study. Thereafter, head-only CO_2 and TED euthanasia are also recommended euthanasia methods, although CO_2 can be aversive, which was not evaluated in the current study, and TED could provide a biosecurity risk due to blood loss.

ACKNOWLEDGMENTS

This work was supported by the U.S. Poultry Foundation/U.S. Poultry and Egg Association (grant BRF011).

DISCLOSURES

No conflict of interest.

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