105 (2026) 106082

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Tool-based euthanasia methods for broiler chicks: Evaluating welfare outcomes of Iris and Kelly scissors

Aaliyah Gore [a], Laya K.S. Alves [b], Gustavo V. Silva [c], Silvia Carnaccini [d], Nathan C. Nelson [a], Sara Reichelt [e], Monique D. Pairis-Garcia [a], Rocio Crespo [a], Allison N. Pullin [f,*]

[a] Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA
[b] Department of Nutrition and Animal Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil
[c] Department of Surgical Specialties and Anesthesiology, Botucatu Medical School, São Paulo State University (Unesp), Botucatu, São Paulo, Brazil
[d] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[e] Aviagen North America (NA), Huntsville, AL, USA
[f] Prestage Department of Poultry Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC, USA

ARTICLE INFO

Keywords:
Euthanasia
Broiler chicks
Cervical dislocation
Tool-assisted euthanasia
Poultry welfare

ABSTRACT

The most common euthanasia method for poultry is manual cervical dislocation (CD), but it is challenging to consistently perform in young chicks due to their small body size. Tool-assisted CD may offer an alternative. Our objective was to evaluate the efficacy of two tool-assisted CD methods with Iris (ICD) and Kelly (KCD) scissors compared to manual CD in 1-day-old (1DOA) and 7-day-old (7DOA) broiler chicks. Chicks were randomly and equally allocated to one of three euthanasia treatments: CD (n=20 1DOA, n=20 7DOA), ICD (n=20 1DOA, n=20 7DOA), and KCD (n=20 1DOA, n=20 7DOA). All euthanasia methods effectively rendered chicks insensible, followed by respiratory and cardiac arrest on the first attempt, with cardiac cessation occurring within 52 to 253 sec. Chicks euthanized with CD achieved cardiac arrest sooner at both ages ($p=0.0154$), displayed less mouth gaping at both ages ($p=0.0006$), lost the pupillary light reflex faster at 1DOA ($p=0.0177$), and displayed shorter clonic convulsions ($p=0.0331$) and feather erection ($p=0.0538$) at 7DOA compared to chicks euthanized with ICD and KCD. However, CD also displayed the highest macroscopic laceration score ($p<0.0001$) and subcutaneous hemorrhaging ($p<0.0001$) than KCD and ICD, and CD was the only method to result in decapitation. The ICD method achieved a similar consistency as CD in the site of vertebral separation, with the majority of separations occurring between C1-C2 vertebrae for both methods ($p=0.0046$). The KCD method resulted in lower separations (C2-C3 vertebrae; $p<0.0001$) and a higher incidence of vertebral and crushed fractures than CD and ICD ($p=0.0303$). Taken together, although all methods were successful in rendering chicks insensible, the CD resulted in insensibility metrics occurring sooner, particularly at 7DOA. However, the CD method demonstrated more lacerations and greater risk for decapitation, which may be more emotionally aversive to the person performing euthanasia. The ICD method offered an alternative to achieve similar consistency in cervical dislocation and brain trauma while minimizing macroscopic lesions and fractures.

Introduction

Euthanasia is defined as "ending an animal's life in a way that minimizes or eliminates pain and distress" and is a recognized practice intended to support animal welfare under specific circumstances (AVMA, 2020). Euthanasia is a critical component of veterinary medicine and ethical farm management. The American Veterinary Medical Association (AVMA) has established policies defining and describing approved euthanasia methods and tools (AVMA, 2020). Each method has its advantages and limitations, influenced by factors such as bird size, technique feasibility, and training requirements for personnel. For chicks, approved euthanasia methods include, but are not limited to, cervical dislocation, maceration, gas inhalation, and decapitation (AVMA, 2020). In commercial hatcheries, gas inhalation or maceration are the most routinely used methods for euthanizing day-old chicks, while manual cervical dislocation is the most common euthanasia method used on farms (Sparrey et al., 2014; Martin et al., 2016; Linares et al., 2018; Nielsen et al., 2019).

* Corresponding author at: 2711 Founders Drive, Campus Box 7608, Raleigh, NC 27695.
E-mail address: apullin@ncsu.edu (A.N. Pullin).

https://doi.org/10.1016/j.psj.2025.106082
Received 31 July 2025; Accepted 7 November 2025
Available online 8 November 2025

Following placement in the grow out house (days 1 to 14), broiler chicks may require euthanasia for a variety of reasons, including severe illness, injury, or failure to thrive (Linares et al., 2018; Nielsen et al., 2019). Cervical dislocation is an AVMA-approved method for young and mature poultry, where proper implementation requires complete vertebral dislocation between the base of skull and C1 vertebra (Linares et al., 2018; Sivula and Suckow, 2018; Nielsen et al., 2019; AVMA, 2020). However, manual cervical dislocation can be challenging to perform on small chicks and risks meeting AVMA standards for euthanasia if performed ineffectively, such as incomplete separation of the vertebrae and skull, crushing of the vertebrae, or unintended decapitation (Woolcott et al., 2018; Nielsen et al., 2019). Incomplete cervical dislocation is a significant animal welfare concern, as it may prolong attaining insensibility and delay death (Martin et al., 2018; Woolcott et al., 2018; Bandara et al., 2019a; Boyal et al., 2020; Baker-Cook et al., 2021). In addition to CD, decapitation is also another AVMA-approved euthanasia method that can be used when performed by competent personnel using a sharp instrument (AMVA, 2020). However, this procedure can be visually challenging for personnel and may cause emotional discomfort for those performing the procedure, indicating the need to evaluate other euthanasia tools (AVMA, 2020).

To address these issues, the poultry industry is exploring improved euthanasia techniques, particularly for scenarios where manual cervical dislocation is frequently performed. Alternative techniques involve the use of mechanical tools to aid in cervical dislocation and improve the consistency and accuracy of the procedure. Existing tools include the Koechner Euthanizing Device (KED) and a novel cervical dislocation tool (NCDT), both of which have shown efficacy in achieving complete spinal column separation when used correctly (Boyal et al., 2020; Ripplinger et al., 2024). However, the NCDT tool was specifically designed for older poultry (>3 weeks of age), and minimal work has been done focusing on tool-assisted cervical dislocation in younger birds (<2 weeks of age). Previous studies have demonstrated that the KED model S designed for chicks and poults may not be recommended as a euthanasia method. The KED-S resulted in a prolonged time to death and no vertebral luxation in 2 to 3-day-old chicks compared to manual cervical dislocation or using a table edge to assist with cervical dislocation (Bandara et al., 2023). In 7- and 21-day-old broilers, the KED-S performed poorly compared to manual cervical dislocation, leading to more severe vertebral fractures and a longer time to insensibility and death (Baker-Cook et al., 2021). Although the KED-S successfully euthanized 3-week-old turkey poults in a previous study, when assessing 1-week-old turkey poults, the KED-S did not result in cardiac arrest within the researchers' five minute endpoint, indicating this tool was not appropriate for young poultry and alternative tools are needed for the industry (Woolcott et al., 2018).

The objective of this study was to investigate the efficacy of two different scissors (Iris and Kelly) as methods for tool-assisted cervical dislocation in 1- and 7-day old broiler chicks compared to manual cervical dislocation, with a focus on their effectiveness, practicality, and welfare implications. These tools were chosen as they are easily accessible, compact, and affordable. Given the sensitivity of this topic, especially among the general public, this research emphasizes transparency, ethical considerations, and adherence to established welfare guidelines. The goal is to provide evidence-based insights that support both humane practices and operational feasibility in commercial poultry settings. By assessing physiological and behavioral indicators, this study contributes to ongoing efforts to refine euthanasia methods in a way that respects both animal welfare and the realities of on-farm management.

Material and methods

The protocol was approved by North Carolina State University (NCSU) Institutional Animal Care and Use Committee (no. 22-482), and animals were treated in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Tucker et al., 2020). Given the absence of data for a power analysis specific to the efficacy of the two tools, the sample size was determined based on ethical considerations.

Animals and euthanasia treatments

A total of 120 female off-sexed cull broiler breeder chicks were sourced from a commercial hatchery and utilized for the study. One week prior to trial initiation, 60 1-day-old chicks were transported from the hatchery to an NCSU research facility, where they were housed in brooder cages for one week with *ad libitum* access to water and feed. These birds served as the 7-day-old treatment group for the trial (7DOA). On the day of trial, an additional 60 1-day-old chicks (1DOA) were transported from the hatchery to the same NCSU research facility where they remained in their chick transfer box until trial initiation.

Both 1DOA and 7DOA chicks were randomly and equally allocated to one of three euthanasia treatment groups: manual cervical dislocation (CD; $n = 20$ 1DOA, $n = 20$ 7DOA), tool-assisted cervical dislocation with Iris scissors (ICD; $n = 20$ 1DOA, $n = 20$ 7DOA), and tool-assisted cervical dislocation with Kelly scissors (KCD; $n = 20$ 1DOA, $n = 20$ 7DOA). All euthanasia procedures were conducted by a poultry veterinarian with over 10 years of industry experience. For the CD procedure, the veterinarian securely held the chick in their non-dominant hand while using their dominant hand to grasp the base of the neck. The 1DOA chicks were restrained by gently holding the body in the non-dominant hand to minimize the risk of leg injury, while the 7DOA chicks were restrained by the legs. With a swift motion, pressure was applied to the base of the skull as the head was retracted dorsally while simultaneously applying a ventrodorsally rotation force, aiming to dislocate the cervical vertebra from the skull, in accordance with AVMA guidelines (2020).The ICD and KCD euthanasia procedures were performed similarly, with the handle of the scissors positioned at the base of the chick's skull and quickly closed without retraction of the head, aiming for separation between the cervical vertebra and the brainstem. The Iris scissors (4.5″ Iris Scissors, Model #48,148, Prestige Medical, Northridge, California) and Kelly scissors (5.5″ Dressing Scissors, Model# 57,157, Prestige Medical Northridge, California) are displayed in Fig. 1.

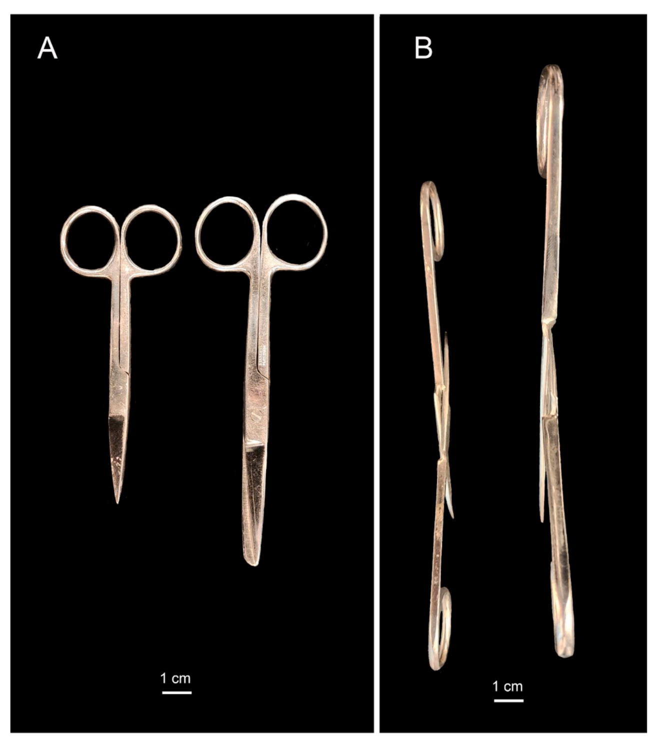

Fig. 1. A) Front view of Kelly and Iris surgical scissors. B) Open view of Kelly and Iris scissors showing the width of the handles.

Evaluation of euthanasia efficacy

Immediately after cervical dislocation, insensibility and loss of brainstem function were assessed using the criteria outlined in Table 1. Death was based on cardiac cessation as determined via stethoscope auscultation (Littmann 3200 Bluetooth Electric, Neuss, Germany). Following confirmation of death, laceration and subcutaneous hemorrhage were macroscopically evaluated according to the criteria in Table 2. Chicks were then transferred to an adjacent room for lateral whole-body imaging with a portable X-ray unit (MasterRad A6 Handheld Portable High-Frequency X-Ray Generator, Medicatech Inc., Irvine, CA; Wireless Flat Panel Detector, Pinnacle X-Ray Inc., Suwanee, GA; Acquisition and Processing Software, Voyance, Irvine, CA). Radiographs ($N = 120$) were captured at a film-focus distance of 76 cm, with radiographic settings of 60 kVp/3 mAs. Images were downloaded as DICOM files and scored by a board-certified veterinary radiologist to evaluate the site of cervical dislocation (Table 3) and the presence of fractures in the vertebral bodies (Table 4) using a modified scoring rubric from Bandara et al. (2019a). Following macroscopic evaluation and radiographs, 8 chicks/treatment were randomly selected for microscopic evaluation of head, spinal column, and dislocation site. Tissue samples were sectioned and fixed in 4 % neutral buffered formalin for 1 week. Attached heads and neck sections were decalcified in 10 % EDTA solution (pH 7.4) at room temperature. After 4 weeks, samples were cut rostro-caudally in 3 to 5 sagittal sections of 0.5 cm thickness. Each section was evaluated for lesions. Microscopic lesions included degree of demyelination, myonecrosis, parenchymal, and subdural hemorrhage and were scored based on parameters in Table 5. Additionally, spinal columns for each bird were cut longitudinally and evaluated for microscopic fractures. Decapitated chicks were not included in the subset randomly selected for microscopic evaluation because decapitation only occurred within the CD group and comparisons between treatment groups could not be made.

Statistical analysis

The response variables included clonic convulsion duration, mouth gaping frequency, cardiac arrest time, feather erection time, latency to lose pupillary light reflex (PLR), laceration score, subcutaneous hemorrhage score, sites of separation (Occipital-C1, C1-2, C2-3, C3-4 and C4-5), degree of separation (luxation and subluxation), and fracture characteristics (sagittal, transverse, articular proc, dens and crushed vertebrae). Data were analyzed using R programming language in RStudio integrated environment (Version 4.2.2), and functions used are represented as package::function. Normality was assessed using histograms (graphics::hist) and the Shapiro-Wilk test (stats::shapiro.test). The physiological variables and scores (clonic convulsion duration, mouth gaping frequency, cardiac arrest time, feather erection time, latency to lose PLR, laceration score, subcutaneous hemorrhage score, and microscopic lesion score) were then modeled using Generalized Linear Models (stats::glm), with age (1DOA and 7DOA), euthanasia method

Table 1
Measures of insensibility and death evaluated following cervical dislocation of 1- and 7-day-old broiler chicks subjected to different euthanasia techniques.

Measure	Description	Procedure
Colonic convulsion	Rapid, involuntary, movement of the body and wings	Visual observation of rapid uncoordinated body movement (wing flapping, foot paddling, leg tremors). Time was recorded when all movement ceased.
Mouth gaping	Quick, irregular inhalation through the mouth	Visual observation of mouth gaping was manually counted until cardiac arrest.
Cardiac arrest	Cessation of heart function	Stethoscope was used to auscultate the heartbeat until the beat was no longer detected.
Feather erection	Complete erection of feathers	Visual observation of feather erection recorded in seconds
Pupillary light reflex cessation	Constriction of the pupil in response to light	A medical grade flashlight was used to shine light directly into the eye every 2-5 seconds. Time was recorded when the pupil constriction reflex was no longer present.

Table 2
Scoring system for macroscopic evaluation of lacerations and subcutaneous hemorrhaging assessed at dislocation site in 1- and 7-day old broiler chicks subjected to different euthanasia techniques.

Measurement	Scoring System
Laceration and external bleeding	0 = no laceration of the skin 1 = laceration of the skin with no external bleeding 2= laceration of the skin with external bleeding
Subcutaneous hemorrhage (SCH) at cervical dislocation site	0 = none 1 = < 25 % of surface area 2 = 26 – 50 % of surface area 3 = 51 – 75 % of surface area 4 = 76 – 100 % of surface area

Table 3
Location and degree of cervical dislocation in 1- and 7-day old broiler chicks subjected to different euthanasia techniques.

Location	Definition
Occipital	Separation occurs between the base of the skull (occipital bone) and C1 vertebrae, where no vertebrae are attached to the occipital bone
C1 (Atlas)	Separation occurs between C1 and C2 vertebrae, where C1 remains attached to the occipital bone
C2 (Axis)	Separation occurs between C2 and C3 vertebrae, where C1 and C2 remain attached to the occipital bone
C3	Separation occurs between C3 and C4 vertebrae, where C1, C2, and C3 remain attached to the occipital bone
C4	Separation occurs between C4 and C5 vertebrae, where C1–C4 remain attached to the occipital bone
Degree	**Definition**
Luxation	Complete dislocation of 2 vertebrae (or C1 and occipital bone) at the articular process joints
Subluxation	Partial (incomplete) dislocation of 2 vertebrae at the articular process joints

Table 4
Location and description of fracture by type in 1- and 7-day old broiler chicks subjected to different euthanasia techniques (modified from Bandara et al., 2019a).

Location	Definition
Crushed vertebral bodies	Multiple fractures of a vertebra that cannot be classified as a fragment, articular process, or dens fracture
Dens	Fracture of the dens (cranial bony projection on C2).
Sagittal	Fracture down the sagittal place of vertebrae
Transverse	Fracture of one or more transverse processes (bony projection located on the dorsolateral aspect of the vertebral body).

Table 5
Scoring system for microscopic lesions assessed at dislocation site in 1- and 7-day old-broiler chicks subjected to different euthanasia techniques.

Measurement	Scoring System
Microscopic lesion score	0 = no lesions 1 ≤ 10 % of surface area affected (mild) 2 = 11-25 % of surface area affected (mild – moderate) 3 = 26 – 40 % of surface area affected (moderate) 4 = 41 – 75 % of surface area affected (moderate-severe) 5 = 76 – 100 % of surface area affected (severe)

(CD, ICD, and KCD), and their interaction as explanatory variables. For chicks euthanized using the manual CD method at 1DOA, additional models were fitted with decapitation status (decapitated or non-decapitated) as an explanatory variable. Radiograph findings were modeled using Generalized Linear Models (stats::glm), then Type III Analysis of Variance (car::Anova) was performed on the fitted models, with age, euthanasia method, and their interaction as explanatory variables. Multiple comparisons were performed using post hoc Bonferroni tests (lsmeans::lsmeans and multcomp::cld). One, 7DOA chick euthanized by KCD was excluded from the radiographic analysis to maintain consistency across groups, as it was the only male in an otherwise all-female population, resulting in $n = 19$ for this group. All statistical tests were performed with significance set at $p < 0.05$.

Results

Assessment of insensibility and death

All chicks were rendered insensible (lack of pupillary light reflex), followed by respiratory and cardiac cessation, on the first attempt across all euthanasia methods, with cardiac arrest occurring within 52 to 253 sec (110.6 ± 39.8, mean ± SD). The euthanasia method had a significant main effect, where chicks euthanized with CD achieved cardiac arrest more rapidly than those euthanized with ICD or KCD ($p = 0.0154$; Fig. 2A). Additionally, age also had a significant main effect, as 1DOA chicks achieved cardiac arrest faster than 7DOA chicks ($p = 0.0001$; Fig. 2A). The interaction between age and euthanasia method was not significant for cardiac arrest ($p > 0.05$).

For signs of insensibility, chicks euthanized with CD displayed fewer mouth gaping events than the chicks euthanized with ICD and KCD at both ages, but mouth gaping frequency in CD was lower for 1DOA chicks compared to 7DOA chicks (age x euthanasia method interaction, $p = 0.0006$; Fig. 2B). At 1DOA, the latency to lose the PLR was shorter with CD compared to ICD; all euthanasia methods at 7DOA resulted in a similar latency to lose the PLR (age x euthanasia method interaction, $p = 0.0177$; Fig. 2C). The 7DOA chicks euthanized with ICD and KCD displayed the longest durations of clonic convulsions compared to 7DOA chicks euthanized with CD, but clonic convulsion durations were similar across euthanasia methods at 1DOA (age x euthanasia method interaction, $p = 0.0331$; Fig. 2D). Similarly, the 7DOA chicks euthanized with ICD and KCD displayed longer durations of feather erection than 7DOA chicks euthanized with CD, but there were no differences between euthanasia methods at 1DOA (age x euthanasia method interaction, $p = 0.0538$; Fig. 2E).

Macroscopic evaluation

The 1DOA chicks euthanized with CD displayed the highest laceration score compared to other euthanasia methods at 1DOA, but all euthanasia methods had similar laceration scores at 7DOA (age x euthanasia method interaction, $p < 0.0001$; Fig. 3A). For subcutaneous hemorrhaging, there was a main effect of euthanasia method, where CD-euthanized chicks again received a higher score compared to ICD- and KCD-euthanized chicks ($p < 0.0001$; Fig. 3B). Age did not affect subcutaneous hemorrhaging ($p > 0.05$). Representative macroscopic findings are displayed in Fig. 4.

Radiographic evaluation

Luxation was more frequent in 1DOA chicks than in 7DOA chicks ($p = 0.0297$) and not affected by euthanasia method ($p > 0.05$; Table 6). Subluxation was not affected by age or euthanasia method ($p > 0.05$). In terms of the location of separation, CD resulted in more frequent separation between C1-C2 than KCD ($p = 0.0046$; Table 6). Separation between C2-C3 was more frequent in chicks euthanized with KCD than ICD ($p < 0.001$), but KCD and ICD were not different from CD. Because some birds displayed separation in more than one cervical site, the sum of values in the results may exceed the number of individuals per treatment Age and euthanasia method did affect the location of separation between Occipital-C1, C3-C4, or C4-C5. Although not statistically different, CD was the only method with separations between C3-C4 and C4-C5.

Transverse and crushed vertebrae fracture types were affected by euthanasia method ($p = 0.0745$ and $p < 0.001$, respectively; Table 7), but differences between methods were not detected in the post hoc test. Both ICD and KCD resulted in crushed vertebrae, with a higher incidence observed in KCD, whereas no crushed vertebrae were observed in chicks euthanized by manual CD. Sagittal and dens fracture types were not affected by age or euthanasia method ($p > 0.05$).

Microscopic evaluation

There were no significant differences in microscopic lesion scores between euthanasia methods or ages ($p > 0.05$; Figure S1). Microscopic evaluation of fractures revealed the KCD method caused a higher incidence of vertebral fractures than CD or ICD ($p = 0.0303$; Table 8). Crushed vertebrae were exclusively observed in the KCD group (Table S1). No significant effects were found for age or the interaction between age and euthanasia method for fractures. Representative microscopic findings are displayed in Fig. 5.

Decapitation

Decapitation was observed in 10 % of 1DOA ($n = 6$) chicks and 1.6 % ($n = 1$) of 7DOA birds, and all decapitations occurred with the CD method. At 1DOA, decapitated chicks displayed a longer duration of clonic convulsions ($p = 0.0428$) and higher incidence of vertebral fractures compared to non-decapitated birds ($p = 0.0218$; Fig. 6); however, these events occurred while the bird was insensible, thus not impacting individual affective state.

Discussion

Cervical dislocation is a commonly used euthanasia method in the poultry industry due to its rapid induction of insensibility and death via cardiac and respiratory arrest. While research has primarily focused on optimizing CD in older birds, there is a notable gap in studies addressing its application in younger chicks (Linares et al., 2018; Martin et al., 2018; Hernandez et al., 2019a; Stiewert et al., 2021). In this study, we sought to evaluate the efficacy of two tool-assisted cervical dislocation methods (ICD and KCD) on 1DOA and 7DOA broiler chicks compared to the manual CD technique. Tool-assisted methods, such as Iris and Kelly scissors, offer a potential solution to challenges associated with manual cervical dislocation in younger birds. For example, these tools may improve precision and consistency in achieving cervical dislocation, thus reducing the risk of incomplete spinal separation or vertebral crushing, which can increase suffering and prolong the duration of sensibility indicators. By focusing on the efficacy of tool-assisted cervical dislocation in young chicks, this study provides valuable insights into a potential refinement of euthanasia practices that could enhance both animal welfare and operational efficiency on farms.

In this study, all euthanasia methods (CD, ICD, and KCD) resulted in insensibility and brainstem failure (i.e., loss of pupillary light reflex, absence of rhythmic breathing, followed by cessation of heartbeat and death) for all birds on the first attempt, regardless of age. The results from this study are similar to previous work by Ripplinger et al. (2024), which demonstrated that tools can be used to assist with cervical dislocation by effectively rendering a bird insensible without a secondary step to achieve death. However, cardiac arrest was achieved sooner for broiler chicks in the current study (110.6 ± 39.8, mean ± SD) compared to 6-week-old broilers in the previous study by Ripplinger et al. (2024) (6-week-old broilers: 222 ± 61 sec, 21-week-old broiler

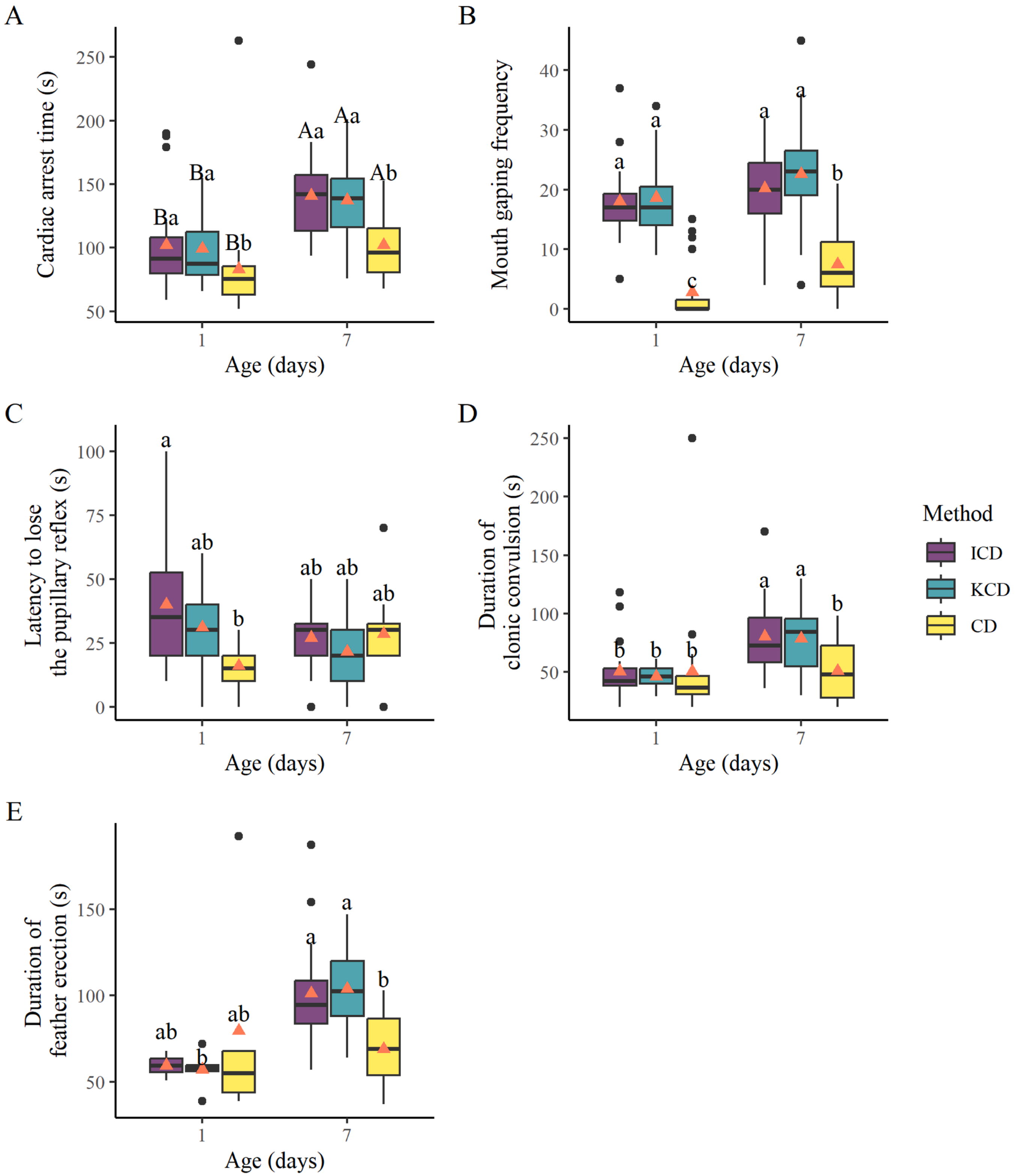

Fig. 2. Comparison of manual cervical dislocation (CD), tool-assisted cervical dislocation with Iris scissors (ICD), and tool-assisted cervical dislocation with Kelly scissors (KCD) for broiler chicks at 1 and 7 DOA for (A) Cardiac arrest time, (B) Mouth gaping frequency, (C) Latency to lose the pupillary reflex, (D) Duration of clonic convulsion, and (E) Duration of feather erection. Uppercase letters indicate within effects ($p < 0.05$, $A > B$) and lowercase letters indicate between effects ($p < 0.05$, $a > b$) where there were no interactions (A). In the cases where there are interactions (B-E), statistical differences are indicated by lowercase letters where $p < 0.05$ ($a > b > c$).

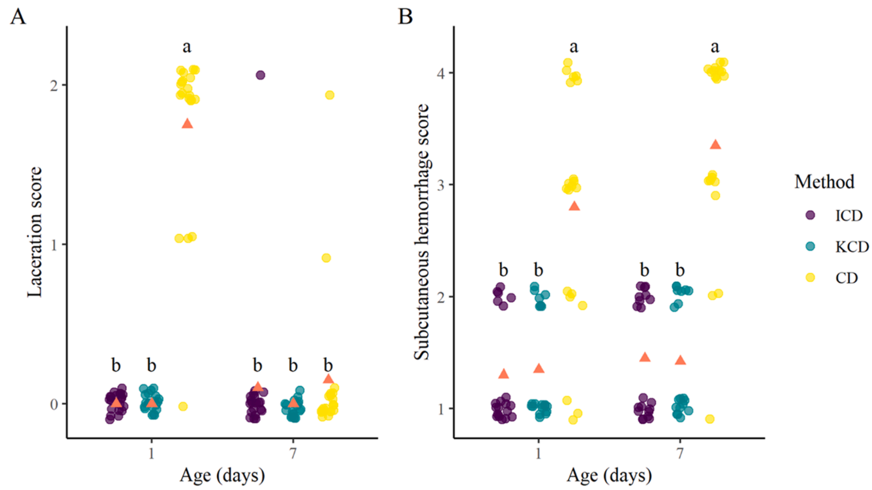

Fig. 3. Comparison of manual cervical dislocation (CD), tool-assisted cervical dislocation with Iris scissors (ICD), and tool-assisted cervical dislocation with Kelly scissors (KCD) for broiler chicks at 1 and 7 DOA for (A) Laceration score and (B) Subcutaneous hemorrhage score. Each dot represents an individual chick, and red triangles represent the mean value for each group. Letters indicate statistical differences where $p < 0.05$ ($a > b > c$).

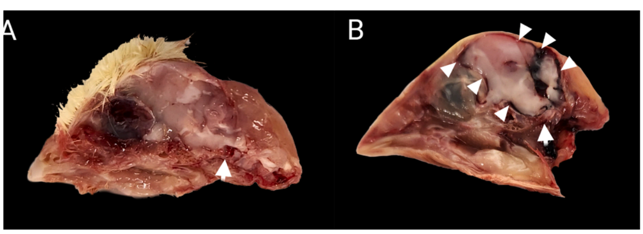

Fig. 4. Representative macroscopic findings in longitudinal sections of chicks' heads from 1 DOA and 7 DOA broiler chicks. A. No macroscopic evidence of subdural or subcutaneous hematomas (score = 0). Note that dislocation is past the third cervical vertebra. The occipital condyle is marked by a white arrow. B. The subdural hematoma reaches the rostral areas of the cranium and is surrounding the cerebellum (white arrowheads; score = 4). Dislocation is present at the occipital condyle (white arrow).

breeders: 92 ± 31 sec, mean ± SD). Our findings coincide with other euthanasia work in cattle, pigs, and other poultry species, in which cardiac arrest occurs more quickly in neonatal animals. This is likely associated with reduced muscle development that results in more significant trauma to the vasculature, thus resulting in heightened sensitivity to sudden changes in oxygen levels (Christensen, 2009; Yair et al., 2012; Begum et al., 2019; Pasquesi et al., 2020).

When assessing cardiac arrest between euthanasia methods in the current study, chicks euthanized with tool-assisted cervical dislocation methods (ICD and KCD) experienced a prolonged time to cardiac arrest compared to CD. These findings align with previous research showing that CD resulted in a quicker cardiac arrest in younger (2- and 3-day old) layer chicks compared to the KED (Bandara et al., 2023). However, these results contrast with work conducted by Ripplinger et al. (2024), who demonstrated that cardiac arrest time was not different between CD-euthanized broilers, broiler breeders, and those euthanized with a novel cervical dislocation tool. Differences between studies may be attributed to the design and force of the tools, as the narrow shanks of the ICD and KCD scissors likely cause less trauma to the surrounding vasculature compared to the physical force used with the novel cervical dislocation tool. Another potential contributing factor may be tissue development, as a mature broiler breeder has more musculature compared to younger chicks, thus impacting the severity of tissue trauma and blood loss. Although animals that are rendered insensible are unable to perceive pain, the AVMA Guidelines for the Euthanasia of Animals (2020) emphasize that death should occur in a timely manner. This is to prevent the potential risk of animals regaining consciousness if a method is incorrectly performed. Therefore, a rapid time to death remains an important welfare consideration, and methods resulting in a prolonged time to death are at a disadvantage from an animal welfare perspective.

In addition to prolonged cardiac arrest, the durations of clonic convulsions and feather erection in 7DOA birds were also longer in the ICD and KCD treatment groups compared to CD. These results agree with previous work that found longer clonic convulsion durations in laying hens euthanized by the KED compared to CD (Hernandez et al., 2019a). Clonic convulsions are involuntary responses and are often the first visual signs after successful cervical dislocation, as they result from neuronal activity following brainstem and spinal cord death (Boyal

Table 6
Degree of separation and location from radiograph evaluation in 1- and 7-day old broiler chicks subjected to different euthanasia techniques.

Method	Age	N[1]	Luxation	Subluxation	Location of Separation[2]				
					OC-C1	C1-C2	C2-C3	C3-C4	C4-C5
CD	1	20	19^a	1	3	16^a	0^{ab}	1	2
KCD	1	20	19^a	1	5	6^b	12^a	0	0
ICD	1	20	19^a	1	3	10^{ab}	8^b	0	0
CD	7	20	20^b	0	2	16^a	1^{ab}	0	0
KCD	7	19	14^b	5	2	8^b	11^a	0	0
ICD	7	20	14^b	6	2	13^{ab}	5^b	0	0

CD: manual cervical dislocation, KCD: tool-assisted cervical dislocation with Kelly scissors, ICD: tool-assisted cervical dislocation with Iris scissors. Letters indicate statistical differences within columns where $p < 0.05$ ($a > b$). Occipital condyle is abbreviated as OC in OC-C1.

[1] For KCD at 7DOA, one male chick was excluded from the radiographic analysis to maintain consistency with the all-female population ($n = 19$).

[2] Some birds exhibited separations at more than one cervical site; therefore, totals may exceed the number of individuals per treatment.

Table 7
Cervical vertebrae fracture types[1] observed radiographically in 1- and 7-day-old broiler chicks subjected to different euthanasia techniques.

Method	Age	N[2]	Cervical vertebrae fracture types[3]			
			Sagittal	Transverse	Crushed	Dens*
CD	1	20	0	0	0	2
KCD	1	20	0	3	10	1
ICD	1	20	0	3	2	2
CD	7	20	0	0	0	1
KCD	7	19	9	9	0	4
ICD	7	20	0	5	1	5

[1] No fractures were observed in the articular process. CD: manual cervical dislocation, KCD: tool-assisted cervical dislocation with Kelly scissors, ICD: tool-assisted cervical dislocation with Iris scissors.
[2] For KCD at 7DOA, one male chick was excluded from the radiographic analysis to maintain consistency with the all-female population ($n = 19$).
[3] More than one fracture type could be scored on each bird.
* Dens = *a* superior projection from the C2 vertebral body, also known as the odontoid process of the axis.

Table 8
Location of separation and presence of fracture(s) from microscopic evaluation in 1- and 7-day old chicks subjected to different euthanasia methods.

Method	Age	N[1]	Location of Separation			Fracture	
			Occipital Condyle	C1	C2	Yes	No
CD	1	8	6	1	1	1	7
KCD	1	8	3	5	0	6	2
ICD	1	8	6	2	0	2	6
CD	7	8	5	3	0	2	6
KCD	7	8	1	7	0	7	1
ICD	7	8	4	4	0	6	2

[1] For KCD at 7DOA, one male chick was excluded from the radiographic analysis to maintain consistency with the all-female population ($n = 19$).

et al., 2022; Watteyn et al., 2022). Prolonged duration of clonic convulsions with ICD and KCD is most likely related to the difference in the severity of tissue and visceral damage between CD and the tool-assisted methods as described above. Feather erection occurs following clonic convulsions and was hypothesized to be an indicator of cardiac arrest (Erasmus et al., 2010). However, when euthanized with CD, cardiac activity was still present during feather erection in turkeys and laying hens and therefore is not a reliable indicator of death (Bandara et al., 2019b; Hernandez et al., 2019b).

In our study, all birds were rendered insensible on the first attempt before crushing was observed, which is consistent with AVMA guidance. These tools may improve precision and consistency in achieving cervical dislocation, thus reducing the risk of incomplete spinal separation or vertebral crushing. Cervical dislocation in conscious poultry must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord (AVMA, 2020). Crushing is only considered acceptable if birds are first rendered insensible.

Mouth gaping is commonly observed following euthanasia as an unconscious response to hypoxic stress in the body (Guntheroth and Kawabori, 1975; Bandara et al., 2023). In our study, mouth gaping had a shorter duration with the CD compared to the ICD and KCD. Additionally, 1DOA chicks euthanized with the CD method exhibited less mouth gaping than CD-euthanized chicks at 7DOA. The observed patterns in mouth gaping frequency are consistent with previous studies, which found that CD resulted in a lower frequency of gasping compared to tool-assisted cervical dislocation methods, such as the KED model-S, used on 2-3 day-old layer chicks (Bandara et al., 2023) and the KED model-C used on 12- to 70-week-old layers (Bandara et al., 2019a). The latter study also reported an age-related effect, indicating that younger layers exhibited a shorter duration of mouth gaping compared to more mature birds. While the reason for the lower mouth gaping frequency in

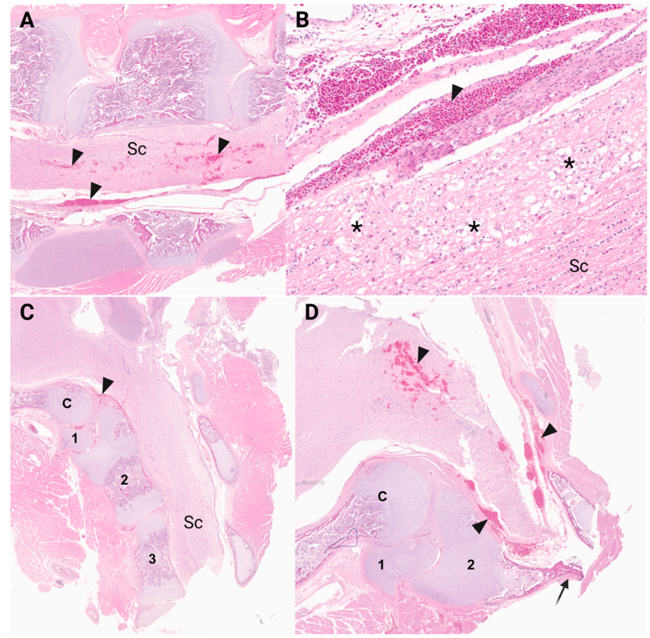

Fig. 5. Representative microscopic findings from 1 DOA and 7 DOA broiler chicks. A-B. Longitudinal sections of the spinal column with spinal cord. A. Subdural, subarachnoid and parenchymal hemorrhages are moderate (black arrowheads, score = 3). B. Higher magnification of the spinal cord (Sc), with hemorrhage (black arrowhead) and demyelination (black asterisks). C. Longitudinal section of posterior cranium with vertebrae attached (dislocation past third cervical vertebra; C – condyle; vertebrae numbered 1-3). Minimal subdural hemorrhage (black arrowheads, score =1). D. Longitudinal section of posterior cranium with fractured vertebrae (black arrow). Moderate local parenchymal and sub-meningeal hemorrhage (black arrowheads, score =3).

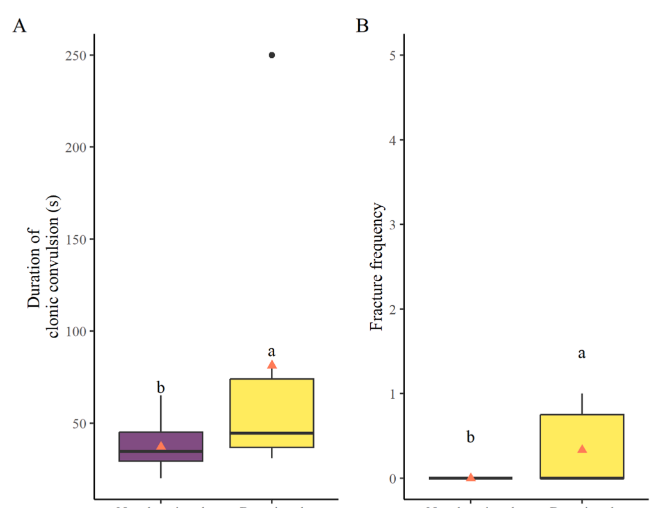

Fig. 6. Comparison of chicks euthanized with cervical dislocation (CD, $n = 20$) at 1DOA that were either non-decapitated (purple; $n = 14$) or decapitated (yellow; $n = 6$) for (A) Duration of clonic convulsions and (B) Fracture frequency (the sum of all fractures). Letters indicate statistical differences where $p < 0.05$ ($a > b$).

younger birds remains unclear, it may be connected to day-old chicks having a greater tolerance for carbon dioxide compared to older birds, resulting in a delayed response to hypoxia (Guntheroth and Kawabori, 1975; Jaksch, 1981). The CD method resulted in the lowest mouth gaping frequency across both age groups, likely due to the increased tissue trauma it causes, leading to more rapid loss of blood and reduced oxygen delivery to tissues.

This study also evaluated the latency to lose PLR as an indicator of brain-stem activity associated with insensibility. PLR is a partially voluntary response in avian species due to skeletal muscle located around the iris (Li and Howland, 1999). Although PLR is partially under voluntary control, previous research indicates that PLR is still a reliable indicator of insensibility (Erasmus et al., 2010; Bandara et al., 2019a, 2023; Stiewert et al., 2021). In the present study, 1DOA chicks euthanized by ICD maintained their PLR twice as long on average compared to the CD-euthanized chicks of the same age. However, the 1DOA ICD group exhibited greater variation PLR duration compared to the other groups. After euthanasia, it was observed that several 1DOA ICD chicks regained their PLR while other sensibility parameters were recorded until cardiac arrest. During the process of death, conditions of hypoxia, or decreased blood flow to the brain stem, avian pupils can become fixed or temporarily dilated. This physiological response to death interfered with the ability to record a constant PLR and was recorded as prolonged PLR because the pupil was not able to restrict until cardiac arrest (Heard, 2000; Erasmus et al., 2010).

As expected, CD caused more macroscopic tissue trauma than ICD or KCD. The scissor handles are narrower and likely more precise than hands when maneuvering between the vertebra and require less force to dislocate the vertebrae and sever the spinal cord. Similarly, CD caused more trauma than the tool-assisted KED method in previous work (Bandara et al., 2019a, 2023; Stiewert et al., 2021). Bandara et al. (2023) also noted that the KED-model-S did not result in brain trauma or spinal cord transaction in 2-to-3-day-old chicks. Our findings indicated that CD, ICD, and KCD had similar microscopic tissue trauma to the brain and spinal cord. However, the CD method was also the only euthanasia method to result in decapitation in 1DOA chicks in the current study. Decapitated birds also demonstrated a longer duration of clonic convulsions, which is likely associated with residual energy stored in the spinal cord and the uncontrolled neural activity caused by extensive trauma to the brain stem and cervical spinal cord (Eichbaum et al., 1975). Greater neuronal damage can prolong convulsions until neuronal death occurs, even though the birds showed no evidence of regaining consciousness or experiencing additional stress associated with the decapitation. Nevertheless, decapitation is often considered less aesthetically pleasing for the person performing the procedure and is generally avoided to safeguard their mental well-being (Martin, 2015; Nielsen et al., 2019; Clarkson et al., 2023). The use of tools to assist with cervical dislocation prevented decapitation from occurring and may be beneficial to implement from a human welfare perspective in addition to bird welfare. Taken together, the ICD and KCD tool-assisted methods required less physical force to induce brainstem and spinal cord trauma similar as CD without the macroscopic tissue trauma or risk of decapitation, which may lessen the emotional and physical strain on personnel performing the euthanasia.

The radiographic results showed differences in the degree of luxation between age groups and the location of separation. It is important to note that 1DOA and 7DOA chicks experienced separation in multiple locations. The majority of the 1DOA chicks had complete luxation across all euthanasia methods, while fewer of the 7DOA chicks displayed complete luxation. The lower incidence in the 7DOA chicks may be attributed to more advanced muscle and bone development in older chicks (Yair et al., 2012). The CD and ICD euthanasia methods most achieved dislocation between C1-C2. In contrast, KCD primarily resulted in dislocation between C2-C3. Causing dislocation closer to the base of skull is hypothesized to lead to more brainstem trauma (Erasmus et al., 2010; Bandara et al., 2023). Furthermore, KCD was associated with the most microscopic vertebral fractures and crushed fractures. Crushed fractures and lower sites of separation with the KCD method may have been related to the tool dimensions, as the Kelly scissors have a wider shank (5.47 mm) compared to the Iris scissors' shank (2.5 mm). Other researchers also reported increased crushed vertebral fractures with KED compared to CD in young chickens (Baker-Cook et al., 2021; Bandara et al., 2023). It is worth noting that the CD method was the only method with separations below the C3 vertebrae, despite being performed by an experienced veterinarian. This finding is most likely related to the stretching force applied during manual cervical dislocation, since the technique relies on operator-applied traction, which may propagate further along the cervical column and result in more distal separations. In contrast, ICD and KCD concentrate the dislocation force at a localized point due to the scissors' shanks, which may explain the absence of separations at these lower cervical sites. These findings highlight the potential risk of performing CD on 1DOA chicks.

It is important to note that discrepancies were observed between fracture results obtained through radiographic imaging and those identified through microscopic histology. These differences may be attributed to the difficulty in interpreting radiographs of young chicks, as incomplete mineralization of the vertebral bodies can obscure the extent of fractures (Farrow, 2008; Sanchez-Rodriguez et al., 2019). In contrast, histological analysis may reveal microscopic fractures in portions of the vertebrae that are not detectable as gross fractures on radiographs.

Conclusion

This study provides insight into the efficacy of alternative tools to assist with cervical dislocation (ICD and KCD) for broiler chicks at 1DOA and 7DOA. All euthanasia methods were effective in rendering chicks insensible and causing death on the first attempt at both ages. The CD euthanasia method ultimately resulted in sooner cardiac arrest, shorter durations of insensibility indicators (i.e., latency to lose PLR, clonic convulsions, feather erection), and lower mouth gaping frequencies, indicating that CD is the most effective euthanasia method. However, CD also had a higher score for macroscopic lacerations and subcutaneous hemorrhaging and a higher incidence of decapitation, which can be traumatic for personnel. Notably, decapitation did not occur with the tool-assisted methods. Between the two tools, the ICD euthanasia method had a lower incidence of fractures and a higher location cervical dislocation (i.e., C1-C2 versus C2-C3) than the KCD method and may be a more appropriate tool as a result. While the findings highlight the importance of proper technique and tool selection in minimizing welfare risks, they also suggest that tool-assisted euthanasia methods may offer an effective alternative to manual cervical dislocation, particularly in younger birds.

CRediT authorship contribution statement

Aaliyah Gore: Writing – review & editing, Writing – original draft, Project administration, Investigation, Data curation. **Laya K.S. Alves:** Writing – review & editing, Investigation, Data curation. **Gustavo V. Silva:** Writing – review & editing, Visualization, Formal analysis. **Silvia Carnaccini:** Writing – review & editing, Methodology, Investigation, Data curation. **Nathan C. Nelson:** Writing – review & editing, Methodology, Investigation, Data curation. **Sara Reichelt:** Writing – review & editing, Resources, Investigation, Conceptualization. **Monique D. Pairis-Garcia:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Rocio Crespo:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Allison N. Pullin:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Disclosures

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Allison N. Pullin reports financial support was provided by Aviagen Inc. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge funding from Aviagen North America to conduct the study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.106082.

References

American Veterinary Medical Association [AVMA]. 2020. AVMA guidelines for the euthanasia of animals: 2020 edition. Accessed Aug. 2024. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.

Baker-Cook, B., Torrey, S., Widowski, T.M., Turner, P.V., Knezacek, T., Nicholds, J., Schwean-Lardner, K., 2021. The efficacy of three on-farm euthanasia methods for broiler chickens throughout the production cycle. Br. Poult. Sci. 62, 638–649.

Bandara, R.M.A.S., Torrey, S., Turner, P.V., zur Linden, A., Bolinder, A., Schwean-Lardner, K., Widowski, T.M., 2019a. Efficacy of a novel mechanical cervical dislocation device in comparison to manual cervical dislocation in layer chickens. Animals 9 (7), 407.

Bandara, R.M.A.S., Torrey, S., Turner, P.V., zur Linden, A., Schwean-Lardner, K., Widowski, T.M., 2023. Efficacy of three different cervical dislocation methods for on-farm killing of layer chicks. Poult. Sci. 102, 102538.

Bandara, R.M.A.S., Torrey, S., Turner, P.V., Schwean-Lardner, K., Widowski, T.M., 2019b. Anatomical pathology, behavioral, and physiological responses induced by application of non-penetrating captive bolt devices in layer chickens. Front. Vet. Sci. 6, 89.

Begum, S., Hussain, D.M., Chakravarty, P., Sharma, A., 2019. Neonatal physiology in animals: a short discussion. Int. J. Chem. Stud. 7, 4334–4335.

Boyal, R.S., Buhr, R.J., Harris, C.E., Jacobs, L., Bourassa, D.V., 2020. Equipment and methods for poultry euthanasia by a single operator. J. Appl. Poult. Res. 29, 1020–1032.

Boyal, R.S., Buhr, R.J., Harris, C.E., Jacobs, L., Bourassa, D.V., 2022. Evaluation of mechanical cervical dislocation, captive bolt, carbon dioxide, and electrical methods for individual on-farm euthanasia of broiler breeders. Poult. Sci. 101 (9), 102000.

Christensen, V., 2009. Development during the first Seven days post-hatching. Avian Biol. Res. 2, 27–33.

Clarkson, J.M., Paraskevopoulou, A., Martin, J.E., 2023. A decade on: where is the UK poultry industry for emergency on-farm killing? Poult. Sci. 102, 102604.

Eichbaum, F.W., Slemer, O., Yasaka, W.J., 1975. Postdecapitation convulsions and their inhibition by drugs. Exp. Neurol. 49, 802–812.

Erasmus, M.A., Lawlis, P., Duncan, I.J.H., Widowski, T.M., 2010. Using time to insensibility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys. Poult. Sci. 89, 1345–1354.

Farrow, C.S., 2008. Veterinary Diagnostic Imaging: Birds, Exotic Pets, and Wildlife. Mosby Elsevier, St. Louis, MO.

Guntheroth, W.G., Kawabori, I., 1975. Hypoxic apnea and gasping. J. Clin. Invest. 56, 1371–1377.

Heard, D., 2000. Perioperative supportive care and monitoring. Vet. Clin. North Am. Exot. Anim. Pract. 3, 587–615.

Hernandez, E., James, F., Torrey, S., Widowski, T., Schwean-Lardner, K., Monteith, G., Turner, P.V., 2019a. Evaluation of brain death in laying hens during on-farm killing by cervical dislocation methods or pentobarbital sodium injection. Front. Vet. Sci. 6, 297.

Hernandez, E., James, F., Torrey, S., Widowski, T., Schwean-Lardner, K., Monteith, G., Turner, P.V., 2019b. Electroencephalographic, physiologic and behavioural responses during cervical dislocation euthanasia in turkeys. Vet. Res. 15, 132.

Jaksch, W., 1981. Euthanasia of day-old male chicks in the poultry industry. Int. J. Stud. Anim. Prob. 2 (4), 203–213.

Li, T., Howland, H.C., 1999. A true neuronal consensual pupillary reflex in chicks. Vision Res. 39, 897–900.

Linares, J.A., Dougherty, S., Millman, S., 2018. 7 - Poultry welfare assessment on the farm: focusing on the individual. In: Mench, J.A. (Ed.), Advances in Poultry Welfare. Woodhead Publishing, pp. 131–148.

Martin, J.E., 2015. Humane Mechanical Methods for Killing Poultry On-Farm. Univ. Glasgow. PhD Diss. https://theses.gla.ac.uk/6634/1/2015MartinPhD.pdf.

Martin, J.E., McKeegan, D.E.F., Sparrey, J., Sandilands, V., 2016. Comparison of novel mechanical cervical dislocation and a modified captive bolt for on-farm killing of poultry on behavioural reflex responses and anatomical pathology. Anim. Welf. 25, 227–241.

Martin, J.E., Sandercock, D.A., Sandilands, V., Sparrey, J., Baker, L., Sparks, N.H.C., McKeegan, D.E.F., 2018. Welfare risks of repeated application of on-farm killing methods for poultry. Animals 8, 39.

Nielsen, S.S., Alvarez, J., Bicout, D.J., Calistri, P., Depner, K., Drewe, J.A., Garin-Bastuji, B., Rojas, J.L.G., Schmidt, C.G., Chueca, M.Á.M., Roberts, H.C., Sihvonen, L. H., Spoolder, H., Stahl, K., Calvo, A.V., Viltrop, A., Winckler, C., Candiani, D., Fabris, C., der Stede, Y.V., Michel, V., 2019. Killing for purposes other than slaughter: poultry. EFSA J. 17, 5850.

Ripplinger, E.N., Crespo, R., Pullin, A.N., Carnaccini, S., Nelson, N.C., Trindade, P.H.E., Reichelt, S., Pairis-Garcia, M., 2024. Efficacy of a novel cervical dislocation tool for humane euthanasia of broilers and broiler breeders. Poult. Sci. 103, 103449.

Sanchez-Rodriguez, E., Benavides-Reyes, C., Torres, C., Dominguez-Gasca, N., Garcia-Ruiz, A.I., Gonzalez-Lopez, S., Rodriguez-Navarro, A.B., 2019. Changes with age (from 0 to 37 D) in tibiae bone mineralization, chemical composition and structural organization in broiler chickens. Poult. Sci. 98, 5215–5225.

Sivula, C.P., Suckow, M.A., 2018. Euthanasia. In: Weichbrod, R.H., Thompson, G.A., Norton, J.N. (Eds.), Chapter 35 in Management of Animal Care and Use Programs in Research, Education, and Testing. CRC Press/Taylor & Francis, Boca Raton, FL.

Sparrey, J., Sandercock, D.A., Sparks, N.H.C., Sandilands, V., 2014. Current and novel methods for killing poultry individually on-farm. Worlds Poult. Sci. J. 70, 737–758.

Stiewert, A.M., Wooming, B., Archer, G.S., 2021. Comparing various euthanasia devices and methods on 8 and 12-week-old turkey hens. Poult. Sci. 100, 101053.

Tucker, C.B., MacNeil, M.D., Webster, A.B., 2020. Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition. Accessed Aug. 2024. https://www.asas.org/docs/default-source/default-document-library/agguide4th.pdf?sfvrsn=56b44ed12.

Watteyn, A., Garmyn, A., Ampe, B., Jacobs, L., Moons, C.P.H., Tuyttens, F.A.M., 2022. Comparison of methods for individual killing of broiler chickens: a matter of animal welfare and on-farm feasibility. Front. Anim. Sci. 3, 892186.

Woolcott, C.R., Torrey, S., Turner, P.V., Chalmers, H., Levison, L.J., Schwean-Lardner, K., Widowski, T.M., 2018. Assessing a method of mechanical cervical dislocation as a humane option for on-farm killing using anesthetized poults and young turkeys. Front. Vet. Sci. 5, 275.

Yair, R., Uni, Z., Shahar, R., 2012. Bone characteristics of late-term embryonic and hatchling broilers: bone development under extreme growth rate. Poult. Sci. 91, 2614–2620.