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Comparison of novel mechanical cervical dislocation and a modified captive bolt for on-farm killing of poultry on behavioural reflex responses and anatomical pathology

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Abstract

An alternative emergency method for killing poultry on-farm is required following European legislation changes (EU 1099/2009), which heavily restricts the use of manual cervical dislocation. This study investigated the kill efficacy of two mechanical methods that conform to the new legislation: (1) a novel mechanical cervical dislocation device; and (2) a modified captive bolt device (Rabbit Zinger^{TM}) and manual cervical dislocation (the control). Killing treatments were applied to broilers and layers at two stages of production (broilers: 2-3 weeks and 5 weeks of age; layers: 12-13 weeks and 58-62 weeks), with a total of 180 birds. Latency to abolition of cranial and behavioural reflexes, as well as post-mortem analysis of the physiological damage produced, were used to estimate time to unconsciousness and assess kill efficacy. The novel mechanical cervical dislocation device was reliable and a practical method for killing poultry on-farm, with 100% kill success ($F_{2,167} = 19.96$, $P < 0.001$) and cranial reflex interval mean durations lasting for 0.0 - 103.0 s post-kill (jaw tone ($F_{2,150} = 13.34$, $P < 0.001$); pupillary ($F_{2,150} = 101.66$, $P < 0.001$); nictitating membrane ($F_{2,150} = 1.61$, $P = 0.191$); and rhythmic breathing ($F_{2,150} = 1.46$, $P = 0.235$) compared to the modified Rabbit Zinger™ (72% kill success rate, 0.3-124.9 s cranial reflex mean durations) and manual cervical dislocation (100% kill success rate, 0.0-119.3 s cranial reflex mean durations). The novel mechanical cervical dislocation device resulted in consistent anatomical damage to the birds (e.g. high dislocation of the neck and severing of the spinal cord) compared to the manual method, despite both having 100% success rate, while the modified Rabbit Zinger™ was difficult to operate and resulted in varied anatomical damage. The novel mechanical cervical dislocation device showed promise as a replacement kill method on farm for poultry.

Keywords

Animal welfare, captive bolt, cervical dislocation, killing, poultry, reflexes

Introduction

Determining the efficacy of on-farm killing methods for individual birds is essential to poultry welfare in both commercial and non-commercial contexts. Poultry may need to be killed on farm or in backyard flocks for several reasons (e.g. in an emergency for small-scale disease control or injury, and for stock management). Emergency killing of large numbers of birds are often controlled by whole-house or containerised gas methods, or birds may be transported for slaughter and then slaughtered using gas or electrical waterbath stunning methods. However, for individual birds on-farm, there are two key methods for killing poultry: (1) cervical dislocation, which is designed to cause death by cerebral ischemia and extensive damage to the spinal cord and brain stem (Bader *et al* 2014; Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory & Wotton 1990; Ommaya & Gennarelli 1974); and (2) percussive devices designed to cause extensive brain damage, resulting in brain death (Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory & Wotton 1990; HSA 2004; Mason *et al* 2009; Sparrey *et al* 2014).

Cervical dislocation methods can be divided into two categories: (1) manual – cervical dislocation of the neck by hand (MCD); and (2) mechanical – cervical dislocation of the neck with the aid of a tool (Gregory & Wotton 1990; HSA 2004; Mason *et al* 2009; Sparrey *et al* 2014). The most common method for despatching poultry on-farm is manual cervical dislocation (MCD) (Mason *et al* 2009), as it is perceived to be humane by users, easy to learn and perform, and does not require equipment. All cervical dislocation killing methods are designed to separate the skull from the vertebral column of the bird (C0-C1 vertebral dislocation), resulting in severing of the spinal cord and/or brain stem and the main blood vessels supplying the brain (Cartner *et al* 2007; Gregory & Wotton 1990; Mason *et al* 2009; Parent *et al* 1992; Veras *et al* 2000). It has been suggested that optimal application also produces a concussive effect on the bird due to trauma inflicted on the brain stem through the action of stretching and twisting (Cartner *et al* 2007; Erasmus *et al* 2010a; Harrop *et al* 2001; Pryor & Shi 2006; Shi & Pryor 2002; Shi & Whitebone 2006). However, both methods of cervical dislocation (but MCD in particular, perhaps because it is more common) have been the subject of welfare concern, as research in the last 40 years has questioned their humaneness and consistency in poultry (Erasmus *et al* 2010a; Gregory & Wotton 1986; Gregory & Wotton 1990), as well as other species (Cartner *et al* 2007; Tidswell *et al* 1987). Some studies have indicated that animals, including poultry, may be conscious for a significant period post-application of cervical dislocation methods (Carbone *et al* 2012; Erasmus *et al* 2010a; Gregory & Wotton 1990) and it has been noted that there is high variability in its application across different relevant groups (e.g. poultry stock-workers, veterinarians, trained slaughtermen) (Mason *et al* 2009; Sparrey *et al* 2014). In response to these concerns, as of January 2013, the use of MCD has been restricted through European legislation (EC 1099/2009) to a maximum of 70 birds/person/day and to birds ≤ 3 kg in weight (European Council 2009). As a result, an alternative method for killing poultry on-farm needs to be identified which conforms to the new legislation and is proven to be effective and humane.

Assessing the effectiveness and humaneness of a kill method is achieved, in part, by determining time to unconsciousness (insensibility) and brain death. Several studies have identified and validated the loss of brain stem (e.g. corneal) and spinal (e.g. nociceptive) reflexes as an indicator of loss of consciousness, and/or brain death in poultry (Erasmus *et al* 2010a; McKeegan *et al* 2013; Sandercock *et al* 2014; Sparrey *et al* 2014), as well as in several other species (Croft 1961; Hellyer *et al* 1991). The loss of pupillary reflex and jaw tone have both been used as indicators of unconsciousness Some studies have also used the cessation of clonic convulsions in poultry (e.g. wing-flapping and leg paddling) as an indication of brain death (Dawson *et al* 2009; Dawson *et al* 2007), as well as cessation of rhythmic breathing (Blackmore

& Delany 1988; Erasmus *et al* 2010a; Grandin 1994). The loss of spinal and brain stem reflexes can be attributed to physical trauma to these areas as well as the specific type and scale of trauma and therefore the killing method employed will affect the time to brain death and loss of consciousness (Close *et al* 2007; Shaw 2002).

This study investigates the kill efficacy of two new or modified mechanical devices designed to kill poultry and compares them with MCD, through assessment of duration of brain stem and spinal reflexes post-application and physiological damage identified through post-mortem examination.

Materials and Methods

Animal housing and husbandry

A total of 180 female chickens were used for the study. The birds were tested in two batches of 90 birds on separate days. In each batch 15 layers and 15 broilers, each divided into two age classes (either 7 or 8 birds per type/age-class depending on the day tested, but always totalling 15 over the two days), were assessed for each of the three killing treatments (N=15). Further details about the birds and their accommodation are provided in Table 1. The sample size was chosen to allow significant differences to be identified in behavioural data which is prone to high individual variation across two bird types and two bird age groups (within type) across three killing treatments. A minimum of 12 birds were calculated to provide sufficient power in the analysis (88%), however an additional 3 birds were used per treatment group in order to compensate for any unsuccessful birds and therefore the loss of valid behavioural data.

Upon arrival all birds were individually weighed and wing-tagged. The birds were housed for a minimum of one week prior to the experiment commencing in order to allow acclimatisation to the new housing environment. All birds were housed in floor pens with wood-shavings litter at lower than commercial stocking density in separate rooms per bird type and age group (Table 1), in order to provide the recommended environmental climate (Aviagen 2009; Hy-Line 2012) for each bird type as well as bespoke environmental enrichments (DEFRA 2002a; DEFRA 2002b). Each pen was constructed from a wooden frame with wire-grid sides and roof (L 1.5 m x W 1.0 m x H 1.5 m); as a result all birds had both visual and auditory contact with other birds within the same room. All birds had *ad libitum* access to feed and water. Temperature was checked daily and all birds were inspected twice daily.

Table 1

Study design

Two novel mechanical poultry killing devices, the Modified Rabbit Zinger (MZIN) and a novel mechanical cervical dislocation gloved device (NMCD), were assessed for their kill efficacy in comparison with each other and a control (MCD). The Rabbit Zinger™ (Pizzurro 2009a; Pizzurro 2009b) is a penetrating captive bolt device originally designed to kill rabbits that uses the stored energy in rubber tubes to drive a penetrating bolt into the head, causing death by extensive irreversible brain damage (DEFRA 2014; Martin 2015) (Figure 1a). The device was modified with permission of the original designer in order to adapt it to the new target species (Figure 1b), however the original function and bolt mechanism of the device was retained. The blue Power Tubes^{TM} (Pizzurro 2009a) were used, which require 177 N to pull the bolt into the cocked position (Sparrey *et al* 2014) and when fired the bolt delivered approximately 11.87 J of kinetic energy. The modifications consisted of three aluminium appendages added to the base of the device in order to secure the bird's head in place between them: two rested either side of the bird's head (over the ears, or auricular feathers) and the third ran down the front of the bird's face between the eyes and over the nostrils and beak (Figure 1c). The appendages were designed to position the bird's head correctly in order to direct the bolt (0.6 mm diameter) into the bird's brain and brain stem. Additional leather washers were added to the bolt, in order to reduce the penetration depth from approximately 3.5 cm to 2.5 cm. The device was also weighted at the bottom in order to counteract the top heaviness of the device when cocked.

Figure 1

The NMCD device (Figure 2) was designed to create a mechanical method for cervical dislocation of poultry which mirrored the technique of the manual method. The device consisted of a supportive glove (SHOWA 370 Multi-purpose Stable Glove^{TM}) designed to support the wrist and hand (and therefore could reduce strain injury in the operator) and a moveable metal insert. The metal insert fingers were designed to fit around the bird's head to create a secure grip, and to move independently from side to side in order to allow adjustment for different sizes of birds (Figure 3). The rounded shape of the metal fingers was designed to aid the twisting motion required to dislocate the bird's neck by enhancing the "rolling action" of the hand. The blunt edge between the two metal fingers (protruding < 1 mm from the fleshy area of skin between the index and middle fingers) provided a hard edge to force between the back of the bird's head and the top of the neck, designed to focalise the force into the desired area (i.e. a dislocation at C0-C1) when the method was applied.

Figure 2

Figure 3

The MCD method was performed following the HSA's guidelines; with the bird held upside down by both legs in one hand, and the bird's head held in the operator's palm with the neck between the index and middle finger of the other hand (HSA, 2004). In one swift movement, the operator pulled down on the bird's head, stretching the neck, while rotating the bird's head upwards towards the back of the neck.

Before this trial commenced, the modified devices had been tested in two previous experiments and were applied to 80 cadavers (10 birds per bird type x age for each killing treatment), and 80 anaesthetised birds (10 birds per bird type x age for each killing treatment) that were subject to detailed electroencephalography (EEG) analysis of electrical brain activity, reflex and behavioural duration analysis and post mortem examination. These confirmed that both the MZIN and MMCD caused tissue damage in the expected way that would be likely to result in death, as well as causing rapid and sustained unconsciousness post device application (Martin 2015).

The three killing treatments were tested on 180 live conscious birds across two bird types and ages, resulting in 15 birds per bird type x age for each killing treatment. Across the two batches a Latin-Square design was used to systematically randomise killing treatment, bird type x age and kill order. Killing treatment was allocated to individual birds so not to confound killing treatment with pen. Birds were killed over 5 days for each batch, with 18 birds killed per day. All killing treatments and post-mortem assessments were applied by one trained and experienced operator. A stepwise approach was in place with end points in place if killing treatments reached a level of failure (< 70%). However, the number of kills which were unsuccessful occurred intermittently throughout the two batches and therefore the pre-defined end point was never reached.

The efficacy of the devices was determined in two ways: (1) durations of reflexes post treatment application; and (2) post mortem examination. Three cranial reflexes (pupillary (Croft 1961), nictitating membrane (Erasmus *et al* 2010c; Heard 2000) and rhythmic breathing (Anil 1991; Erasmus *et al* 2010a)) and four relevant involuntary behaviours (presence of jaw tone (Erasmus *et al* 2010a; Sandercock *et al* 2014), cloacal movement (Erasmus *et al* 2010c), and clonic wing flapping and leg paddling (Blackmore & Delany 1988; Erasmus *et al* 2010c; Gregory 1991)) (Table 2) were assessed as present or absent in 15 s intervals post killing treatments application, until an uninterrupted 30 s of absence of all behaviours and reflexes was observed. Assessment of the presence and absence of the behaviours and reflexes was conducted by two observers for all birds: observer 1 assessed reflexes and behaviours associated with the bird's head, while observer 2 assessed measures relating to the body and limbs of the bird. Measures were recorded in a predetermined order for each observer, and using the 1-0 sampling technique (Martin & Bateson 2007): if a reflex/behaviour was present during any point of a 15 s interval it was defined as present for the entire interval, providing a conservative measure of reflex/behaviour duration post killing treatment application. If a reflex or behaviour could not be recorded (e.g. pupillary reflex – concealed due to damage to the eye) the data was recorded as missing.

Table 2

Post-mortem assessment was performed on every bird immediately after all behaviours and reflexes had ceased and the bird was confirmed dead. Specific post-mortem measures were obtained for particular killing treatments as their target areas were different causing damage in different body regions. For all killing treatments, binary yes/no measures were recorded for the presence/absence of the skin being broken, external blood loss and subcutaneous hematoma.

For MZIN, seven specific post-mortem measures were recorded: skull penetration location (see Figure 4 for classified skull regions); a four-scale grading of damage (Table 3) to the left forebrain, right forebrain, cerebellum, midbrain and brainstem; and a binary measure (yes/no) of the presence of an internal brain cavity hematoma.

Figure 4

Table 3

For cervical dislocation killing treatments, seven specific post-mortem measures were assessed. Four binary measures (yes/no) were recorded for dislocation of the neck, vertebra damage (e.g. intra-vertebra dislocation/break), damage to neck muscle, and whether the spinal cord was severed. The level of cervical dislocation was recorded (e.g. between C0-C1, C1-C2, C2-C3, etc.), as well as a measurement of the length (cm) of the gap between the dislocated cervical vertebra. The number of carotid arteries severed (0, 1, or 2) was also noted.

Kill success was defined as only one application attempt with no signs of recovery (e.g. sustained and/or return of rhythmic breathing and jaw tone, for example). If any signs of recovery continued for 15 s (i.e. 1 interval measure) the bird was immediately emergency euthanised; the method of euthanasia was killing treatment dependent in order to prevent post mortem examination data being voided (e.g. for MCD and NMCD it was by the CASH Poultry Killer .22 (CPK 200 – 1 grain (65 mg) gunpowder cartridge) (Accles & Shelvoke 2010); for MZIN it was by MCD. Device success was defined as the killing treatments producing the optimal trauma to the bird, specific to the treatment's design. For example, the MZIN penetrating the skull and causing more than one region of the brain a minimum of "mid" range damage, as pilot work established this was sufficient to result in a successful kill. For the MCD and NMCD, device success was defined as full dislocation of the neck at C0-C1, the spinal cord and both carotid arteries severed and no tears or breaks to the skin (HSA 2004).

Ethical statement

This project was performed under Home Office (UK) authority via Project and Personal Licences and underwent review and approval by SRUC's ethical review committee. All routine animal management procedures were adhered to by trained staff. To protect bird welfare, emergency euthanasia endpoints were in place and adhered to if required.

Statistical Analysis

All data was summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat (14^{th} Edition). Statistical significance was termed by a threshold of 5% level and based on F tests. A P value ranging from >0.05 - <0.10 was defined as a statistical trend. Summary graphs and statistics were produced at the bird level. Statistical comparisons for kill success and device success were conducted via Generalised Linear Mixed Models (GLMMs), using the logit link function and binomial distribution.

Post-mortem measures were divided into neck damage methods (i.e. NMCD and MCD) and head damage methods (MZIN) and analysed separately. Statistical comparisons were performed on sub-sets of data to remove failure birds (i.e. kill success "no") in order to prevent data skewing. All post-mortem binary measures (e.g. skin break yes/no) and categorised measures (e.g. brain damage grade) were analysed via GLMMs using logit link function and binomial distribution. Device success was used as a fixed effect within all the models.

For the reflex/behaviour durations, statistical comparisons were performed on successfully killed birds only, in order to prevent data skewing. The presence/absence of each reflex and behaviour was summarised into interval counts (e.g. present in 0-15 s = 1 count), therefore summarising the data into means of the maximum interval counts at the bird level for each reflex, which were then converted back into the time dimension (s). GLMMs with logit link function and Poisson distributed errors were fitted to the interval counts. Overall statistical comparisons across the killing treatments were conducted. Further analysis involved sub-setting the data into two groups: (1) NMCD and MCD; and (2) MZIN, which allowed post-mortem effects to be fitted into the models as factors. Device success was used as a fixed effect within all the models.

For all models the random effects included the batch, date and the bird ID. All fixed effects were treated as factors and classed as categorical classifications and all interactions between factors were included in maximal models.

Results

A total of 163 out of 180 birds were killed successfully by one of the three methods. Kill success ($F_{2,167}$ = 19.96, P < 0.001) and device success ($F_{2,167}$ = 7.33, P < 0.001) were affected by killing treatments, with NMCD and MCD achieving 100.0 ± 0.0% kill success rate and the MZIN achieving 71.7 ± 5.9% (i.e. 17 birds were not killed successfully by the MZIN) . Device success rates were NMCD = 41.7 ± 6.4%; MZIN = 70.0 ± 6.0%; and MCD = 26.7 ± 5.8%. Kill order had no effect on kill or device success. Bird type had an effect on device success ($F_{1,167}$ = 9.55, P = 0.002), with device success being higher in broilers compared to layer birds, but there was also an interaction between bird type and killing method ($F_{1,167}$ = 4.23, P = 0.036) with device success higher in the MZIN applied to broilers (Figure 5). Bird type had no effect on kill success, although there was a significant interaction between killing treatments and bird type for kill success ($F_{2,167}$ = 3.29, P = 0.040) with the lowest kill success for layer type birds killed by MZIN compared to broiler types, and with remaining killing treatments equally successful for killing (100%), irrespective of bird type. Bird age, kill weight and all other interactions had no significant effects on kill success or device success.

Figure 5

Of the birds killed successfully, means of the maximum duration times for cranial reflexes are shown in Figure 6. Figures 6a and 6c demonstrate that there were no significant differences between killing treatments in relation to mean of the maximum durations for nictitating membrane and rhythmic breathing, but there was for pupillary reflex ($F_{1,150}$ 101.66, P < 0.001) (Figure 6b), in which MZIN showed shorter maximum durations compared to NMCD and MCD birds. Bird type ($F_{1,150}$ = 4.82, P = 0.030), and bird age ($F_{1,150}$ = 6.10, P = 0.015) had an affect on maximum pupillary durations, with layer (33.5 ± 2.5 s) and older (40.2 ± 5.7 s) birds showing higher maximum pupillary durations compared to broilers (27.0 ± 2.2 s) and younger (22.5 ± 3.8

s) birds. Device success (yes or no) had an effect on pupillary maximum duration times (yes: 20.1 ± 2.5 s; no: 39.0 ± 1.8 s) ($F_{1,150}$ = 6.10, P = 0.015) and a tendency to affect nictitating membrane maximum durations (yes: 2.3 ± 1.0 s; no: 3.6 ± 0.9 s) ($F_{1,150}$ = 3.86, P = 0.051), with both showing shorter maximum duration times for birds in which device success was achieved. Nictitating membrane maximum durations were also affected by bird weight ($F_{1,150}$ = 5.09, P = 0.025); and interactions between killing treatments and bird type ($F_{2,150}$ = 5.19, P = 0.007); and bird age and bird weight ($F_{2,150}$ = 7.04, P < 0.001), with heavier (3.3 ± 1.0 s), older (1.96 ± 1.1 s) and layer (3.6 ± 1.0 s) birds showing longer maximum durations compared to lighter (2.7 ± 1.0 s), younger (0.0 ± 0.0 s) and broiler (2.8 ± 1.0 s) birds.

Figure 6

For birds killed successfully, treatment affected the maximum durations of leg paddling, and cloacal movement, but not wing flapping (which ranged 99-113 s). For leg paddling and cloacal movement the NMCD device had the shortest mean of the maximum duration times (97.5 ± 5.6 s, 103.0 ± 6.1 s respectively) compared to the MCD (115.8 ± 6.8 s, 119.3 ± 6.9 s) and MZIN (112.7 ± 7.1 s, 124.9 ± 6.3 s). Leg paddling, wing flapping and cloacal movement were all affected by bird type and bird age (Table 4), with broilers and younger birds having shorter maximum duration times compared to layers and older birds (Table 5). For cloacal movement duration, bird weight also had an effect, with heavier birds exhibiting longer durations (113.5 ± 7.5 s) compared to lighter birds (96.1 ± 9.8 s).

Table 4

Table 5

MZIN (0.3 ± 0.3 s) had significantly the shortest jaw tone duration compared to the NMCD and MCD (8.8 ± 1.3 s; and 6.8 ± 1.3 s, respectively) (Table 4), but there was no significant difference between the MCD and NMCD. Device success, bird type, bird age and bird weight did not significantly affect jaw tone maximum durations. However, the interactions between kill treatment and bird type; kill treatment and bird age; and bird age and kill weight were shown to have an effect. The key differences relating to the interaction between kill treatment and bird type were that the MZIN and NMCD showed that broilers had shorter jaw tone durations (6.5 ± 1.7 s) compared to layers (11.0 ± 1.8 s), but the MCD showed no differences between bird types (broiler = 6.5 ± 1.7 s; layer = 7.0 ± 1.9 s). The interaction between bird age and kill treatment demonstrated that for the MCD and MZIN there were no differences between different bird ages on jaw tone maximum durations. For the NMCD broiler chicks had the shortest jaw tone durations (3.0 ± 1.6 s versus 8-14 s), but layer pullets were shown to have the longest durations (14.0 ± 3.1 s), while broilers (slaughter age) and layer hens had no significant differences (range 8-10 s).

The percentage of successfully-killed birds that exhibited various reflexes and involuntary behaviours varied by killing treatments, although the MCD and NMCD were similar (Table 6).

For nictitating membrane and pupillary reflexes, both the MCD and NMCD had numerically higher percentages of birds displaying these reflexes post-kill compared to MZIN, but these were not significant. However, the MZIN was the only killing treatment in which a single bird showed rhythmic breathing following a successful kill. In all killing treatments the majority of birds displayed convulsive behaviours post-application (e.g. wing flapping and leg paddling) and the last behaviour to cease was cloacal movement. Cloacal movement was not observed in a small number of birds (7 birds of successful kills), however this was due to the birds defecating and the movement being hidden as a result.

Table 6

Both the NMCD and the MCD caused subcutaneous hematomas in the neck, damage to the neck muscle, cervical dislocation and spinal cord severance in 100% of successfully-killed birds (n = 60). A small proportion of birds showed minor tears to the skin (MCD – 6.7%; NMCD – 8.3%), with fewer exhibiting external blood loss from the wounds (both 5%). There were no significant effects of killing treatments on skin tears ($F_{1,103}$ = 0.12, P = 0.732) or external blood loss ($F_{1,103}$ = 0.00, P = 0.978). There was no significant difference between the NMCD and MCD in terms of dislocation position ($F_{1,103}$ = 0.79, P = 0.376), with a C0-C1 dislocation level achieved in 85% of birds for NMCD and 80% for MCD. The MCD attained the lowest break at C3-C4 in one bird. Bird type ($F_{1,103}$ = 32.00, P < 0.001) and bird age ($F_{1,103}$ = 32.14, P < 0.001) had significant effects on dislocation level, with layers and older birds more likely to be subject to lower dislocations (≥ C1-C2) compared to broilers and younger birds. Dislocation level had no effect on the maximum durations for all reflexes and behaviours.

The NMCD caused 0% vertebrae damage as a result of the dislocation, but the MCD caused damage in 3.3% of birds, however the difference was not significant ($F_{1,103}$ = 2.02, P = 0.158). There was an interaction between killing treatments and bird age ($F_{2,103}$ = 4.43, P = 0.038), with two hens killed by the MCD method receiving damage to their vertebra.

Gap distance between the two points of dislocation was significantly affected by killing treatments ($F_{1,103}$ = 7.65, P = 0.007) and bird weight ($F_{1,103}$ = 25.39, P < 0.001). The NMCD method was more likely to result in a larger gap distance compared to the MCD (6.29 ± 0.27 cm and 5.47 ± 0.21 cm respectively). Heavier birds were more likely to have large neck gap distances compared to lighter birds (6.8 ± 0.38 cm and 4.9 ± 0.41 cm respectively). Bird type, bird age, dislocation level and all interactions did not affect gap distances (data not shown). The maximum neck gap sizes for each killing treatments were 9.0 cm for MCD and 10.0 cm for NMCD.

The number of carotid arteries severed was affected by killing treatments ($F_{1,103}$ = 4.58, P = 0.030), with the NMCD more likely to sever ≥1 carotid arteries compared to the MCD (means: NMCD = 1.22 ± 0.11; MCD = 0.90 ± 0.11). The NMCD resulted in 71.7% of birds having ≥1 carotid arteries severed, compared to the MCD where only 58.3% of birds had ≥1 carotid arteries severed. The number of carotid arteries severed was also affected by neck gap distance ($F_{1,103}$ = 22.05, P < 0.001), with larger neck gap distances being positively associated with more carotid arteries being severed. Bird type, age, weight and dislocation level did not affect the number of carotid arteries severed (data not shown). The number of carotid arteries severed did not have a significant effect on maximum durations of any of the reflexes and behaviours measured, apart from having a tendency to affect jaw tone ($F_{2,102}$ = 2.53, P = 0.095), in which severing zero or one carotid artery did not affect maximum jaw tone durations (0 carotid arteries severed: MCD 7.2 ± 2.0 s and NMCD 9.7 ± 2.2 s; 1 carotid artery severed: MCD 8.4 ± 2.3 s and NMCD 12.6 ± 2.3 s), but if two were severed there was a reduction in maximum jaw tone duration (MCD 4.7 ± 2.3 s and NMCD 6.5 ± 2.3 s).

MZIN caused trauma to the head of the bird rather than the neck, therefore comparisons of post-mortem trauma with NMCD and the MCD are not relevant. Kill success did not have significant effect on broken skin, external bleeding and subcutaneous hematomas, with over 88% of birds displaying these factors irrespective of kill success (Table 7). There was an effect of kill success on skull damage ($F_{1,43}$ = 3.21, P = 0.024), with more damage caused with successful kills, but there was no effect in terms of where the skull was penetrated by the bolt ($F_{1,43}$ = 0.19, P = 0.664). Device success had an affect on the location of bolt penetration into the skull, with birds which achieved device success being more likely to have their skulls penetrated at locations CB and CM (Figure 4); 79.1% of birds had damage in these two areas of the skull. The bird type, age, weight and all interactions did not have an affect on the skull penetration area (data not shown).

Table 7

Irrespective of kill success, 64% of birds sustained an internal brain cavity hematoma after application of MZIN (Table 7). Kill success had an affect on the presence of an internal brain cavity hematoma ($F_{1,43}$ = 5.57, P = 0.018), with successfully killed birds more likely to have bleeding within the skull. Device success, bird type and all interactions did not have significant effects. Bird age ($F_{1,43}$ = 16.47, P <0.001) and weight ($F_{1,43}$ = 19.09, P < 0.001) had effects on tissue damage, with heavier and older birds more likely to have internal brain cavity hematomas, compared to lighter and younger birds.

More than 80% of birds killed successfully with the MZIN had damage (low mid or max) to all main areas of the brain (Table 7 and Figure 7), excluding the brain stem, which was damaged in just over 50% of birds. Kill success affected whether or not a brain region was damaged and the grade of the damage. Damage to both sides of the forebrain, the cerebellum, and brain stem was not affected by other factors (e.g. bird type, age, weight, interactions). Bird type had an effect on damage to the midbrain, with layers more likely to sustain damage than broilers ($F_{1,43}$ = 6.03, P = 0.014). Only in successfully-killed birds did the highest grade of damage occur (max), with the cerebellum sustaining the highest proportion of maximum damage. Following unsuccessful kills, less than 45% of birds sustained brain damage and the brain stem was never damaged.

Discussion

This study evaluated the kill efficacy of three killing methods (MCD, NMCD, and MZIN) on broilers and layers at two stages of production. Determining the kill efficacy of on-farm killing methods involves three main considerations: reliability, humaneness and practicality. The NMCD device and the MCD had kill success rates of 100%, compared to the 72% success rate of the MZIN, and therefore were deemed the most reliable methods in this study. Other studies have also demonstrated the high kill success rate in cervical dislocation methods (Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory & Wotton 1990). Erasmus and colleagues (2010a) showed that 100% of turkey hens (N = 26) were successfully killed by mechanical cervical dislocation, re-enforcing the reliability of this method for killing poultry on-farm, but all of those birds displayed a nictitating membrane reflex immediately post device application and maintained this reflex for a mean of 106 s. However, the authors used a Burdizzo (a mechanical cervical dislocation device), which is different to MCD and the NMCD, as it causes dislocation via crushing, not through stretching and twisting (Erasmus et al 2010a). Crushing injury caused by mechanical cervical dislocation methods is a cause for welfare concern as birds may die of asphyxiation rather than cerebral ischemia, resulting in signs of consciousness for longer (Gregory et al 1990). The use of the nictitating membrane as an indicator of insensibility has been questioned, but it has been shown to be a more reliable indicator of complete brain death (Anil 1991; Heard 2000; Sandercock *et al* 2014). Here, no more than 10% of birds ever showed this reflex for any of the three killing treatments and the mean duration of those that did was > 5 s, suggesting that brain death occurred rapidly post-killing treatment application. Whether this is rapid enough to be deemed humane is open to debate.

When the NMCD and MCD were applied, they did not require precision aiming, unlike the MZIN, which meant that a kill success was easier to achieve. MCD does not require any equipment and once trained is relatively simple to apply on birds under 3 kg (HSA 2004). The NMCD glove provided the correct position to hold the bird's head in place to perform the stretch and twisting action, which for an inexperienced individual may be beneficial. Therefore the presence of the glove did not hinder the application of the technique, as both MCD and NMCD had 100% kill success rate. All birds that underwent MCD or NMCD immediately wing flapped and leg paddled vigorously post-application and an obvious internal gap in the neck, between two cervical vertebrae could be felt.

Despite the optimal kill success rate for the MCD and the NMCD, the device success rates were significantly lower compared to that of the MZIN. With the MZIN, only 43/60 (72%) of birds were successfully killed but 42 of those birds also achieved device success, therefore when the method was applied correctly, it achieved an optimal effect on the bird. However, unsuccessful killing of 28% of birds by the MZIN means that, despite its device success when it does kill, it is an unacceptable method for killing poultry. Device success was greatly reduced for layer-type birds compared to broilers for both the MCD and NMCD, which may be due to the more mature skeleton and anatomy of the layer birds compared to the broilers, which would have made it more difficult to dislocate the neck at higher points (e.g. C0-C1 or C1-C2), and therefore more difficult to sever the spinal cord and carotid arteries, as with increasing age these vertebrae become fused to the base of the skull and there is development of fibrous connective tissue around it (McLeod *et al* 1964). MCD performed worst in terms of device success (27%) due to the lower percentage of birds having both carotid arteries severed and fewer birds showing a dislocation level of C0-C1 compared to the NMCD. Severing of one or more carotid arteries causes a reduction in blood flow to the brain (Aslan *et al* 2006; Perry *et al* 2012; Whittow 2000) and results in a reduction of arterial pressure and eventual cerebral ischemia and/or hypoxia (Gregory & Wotton 1986; Gregory & Wotton 1990). However, even if the carotid arteries were not completely severed, the stretching trauma results in narrowing and occlusion of the carotid arteries which may have the same effect as severing them (LeBlang & Nunez 2000a; Whittow 2000). Both NMCD and MCD caused trauma to both carotid arteries, although did not always sever them. This suggests that blood supply to the brain would be rapidly and significantly reduced (LeBlang & Nunez 2000b; Perry *et al* 2012; Weir *et al* 2002), resulting in inability in the brain to function correctly and the onset of neurogenic shock (Dumont *et al* 2001a), which could be inferred as the bird not being fully conscious or suffering vasovagal episodes, as seen in human cases of severe blood loss or restriction (Day *et al* 1982). Previous work has also demonstrated that the higher up the carotid arteries are severed (e.g. at C0-C1 rather than C3-C4), the less likely that false aneurysm formations and early arrested blood flow occurs (Gregory *et al* 2012), both which could elongate the time to brain death. Several studies have also highlighted the importance in severing both carotid arteries in exsanguination methods for poultry as well as other livestock species in order to minimise the duration of brain activity (Blackman *et al* 1986; Gregory *et al* 2012; Raj *et al* 2006). The same trauma should also reduce the blood supply to the top of spinal cord, which causes functional impairment and could result in neurogenic shock (Dumont *et al* 2001a; Dumont *et al* 2001b). The requirement to sever both carotids may not be necessary to ensure that the 'device' or method can be considered successful, providing sufficient stretching and twisting occurs, resulting in blood flow reduction to the brain. The aim to achieve dislocation of the neck at C0-C1 was to ensure the damage and severing of the spinal cord occurred very near to or at the brain stem, enhancing the likelihood of concussion resulting in disruption to brain stem function and localised temporary or permanent biochemical changes within the neural axons (Brieg 1970; Erasmus *et al* 2010b; Freeman & Wright 1953; Krause *et al* 1988; Povlishock *et al* 1992; Takahashi *et al* 1981). More than 80% of birds killed with both MCD and NMCD achieved a C0-C1 dislocation, so the likelihood of trauma to the brain stem was high. Gregory & Wotton (1990) demonstrated that 6/8 birds culled by manual cervical dislocation with dislocation at C0-C1 displayed a reduction in their visual evoked responses, suggesting a loss of consciousness . The results of this study have demonstrated the importance of attempting to sever both carotid arteries and dislocating as near to the skull as possible (e.g. C0-C1), but that the stretch and twist damage was sufficient to kill the bird and minimise the duration of consciousness-indicating reflexes post application (e.g. jaw tone, nictitating membrane, and rhythmic breathing). Therefore the requirements for ‘device success’ may have been too strict in terms of resulting in a humane death, but could be be used as guidance (i.e. gold standard) for optimal performance.

The damage caused by the MZIN to the bird’s head resulted in primary and secondary brain injuries; causing brain contusions, haemorrhaging and axonal damage, all of which disrupt brain function and can cause brain death (Claassen *et al* 2002; Kushner 1998; White & Krause 1993). Successful kills by the MZIN resulted in extensive trauma to the forebrain and the cerebellum. This affected the functioning of several systems e.g. motor systems (unconscious and conscious), cognition, respiration and reflexes (Whittow 2000). The extent of axonal damage is correlated with the amount of the brain damaged (Krause *et al* 1988; White & Krause 1993), therefore the more extensive the brain damage, the more axons are damaged. Axonal damage has also been linked to the length of concussion and unconsciousness (Kushner 1998; White & Krause 1993). Skill was required to aim the device and successful judgment in applying reasonable force in order to prevent the device re-coiling, as well as securing the bird's head in place. If this was not achieved there was a reduction in the penetration depth of the bolt, which resulted in insufficient brain damage to cause death. This is highlighted by the result that approximately 42% of birds which were unsuccessfully killed by the device did not sustain any skull damage, as the head was either missed completely or only a glancing blow was sustained, which caused only soft tissue damage to the neck or eyes; or recoil resulted in insufficient power to penetrate the skull. The MZIN required two operators, one to hold the bird, and other to cock and aim the device, as well as a hard surface to rest the bird on, which could be deemed impractical in an on-farm situation. There was also a health and safety concern with the device, as it is a captive bolt and therefore great care is required during its use, and as such safety equipment must be worn (e.g. gloves, safety goggles) (Pizzurro 2009a; Pizzurro 2009b). However, the primary issue with the MZIN device was its low kill success rate of 72%, which is not reliable enough for a routine on-farm killing method.

Durations of reflexes have been used and validated for inferring consciousness in killing assessments of several animals, including poultry (Erasmus *et al* 2010a; Erasmus *et al* 2010b; McKeegan *et al* 2013; Sandercock *et al* 2014). There were no significant differences between killing methods on durations of rhythmic breathing and nictitating membrane and both were lost within 3.4 s post-kill, suggesting both brain death and therefore unconsciousness occurred rapidly for all killing methods. Loss of pupillary reflex is used as a conservative measure for brain death and complete insensibility (Erasmus *et al* 2010c; Heard 2000; Sandercock *et al*

2014), and the MZIN had the shortest durations for pupillary reflex compared to NMCD and the MCD, however this only occurred in birds killed successfully with the MZIN which was low. Such low reliability of successful kills means that the MZIN cannot be considered to be humane. The shorter duration of the pupillary reflex for the MZIN may be explained by the type and location of trauma the kill treatment caused. The bolt of the MZIN damaged the midbrain in more than 80% of birds; the midbrain is reported to be the area within the brain that controls the nictitating membrane, as well as the pupillary reflex (Solomon 1990; Whittow 2000), therefore direct trauma to it would result in impairment of these reflexes. Damage to the surrounding areas of the brain could also cause indirect trauma to the midbrain (e.g. contrecoup damage) and therefore impair reflexes (Drew & Drew 2004; White & Krause 1993). Mature layer hens (irrespective of age) exhibited longer durations for pupillary reflex when killed with MZIN compared to broilers, which could be attributed to their larger size and more mature anatomy (e.g. fused skulls) of these birds (Hogg 1982), therefore more extensive trauma may be required to cause rapid loss of reflexes. Furthermore, the pupillary reflex is affected by disruption to the blood supply of the retina (e.g. severing of carotid arteries), therefore observed dilation and constriction of the pupil may not be due to a genuine reflex to the light, and thus the pupillary reflex durations for the NMCD and the MCD may be inadvertently elongated (Bilello *et al* 2003; Gregory & Wotton 1990; Perry *et al* 2012; Sharma *et al* 2005). However, it is important to note that more than 75% of all birds across all killing methods showed pupillary reflex in the first 15 s post-application of a kill treatment, suggesting that none of the devices caused immediate brain death.

The MZIN was associated with significantly shorter jaw tone durations than NMCD or MCD, which has been used as an indicator of consciousness (Croft 1961; Erasmus *et al* 2010a; Erasmus *et al* 2010c), suggesting that MZIN caused birds to lose consciousness faster than the other two killing methods, when successful. In broilers, NMCD resulted in shorter jaw tone durations compared to MCD and there was a significant effect of bird age (which was confounded with bird type, as all broilers were less than 5 weeks of age, despite being heavier than mature layer hens). This could be explained by the fact that late production broilers and mature layer hens were heavier birds and therefore have a greater volume of blood and larger blood vessels, which could make it more difficult to stop or minimise blood flow to the brain stem, which controls jaw tone (Solomon, 1990; Whittow, 2000). MCD and NMCD did cause sufficient damage to the brain stem across all birds, demonstrated by short mean durations for jaw tone, as well as less than 40% of birds ever showing the reflex. Sandercock and colleagues (2014) showed that unconsciousness induced by anesthetic was associated with loss of jaw tone in layers and turkeys and was a consistent measure of loss of consciousness in this context. For birds which did not lose jaw tone immediately post device application, there is concern that the birds may be conscious, however the absence of other reflexes alongside (e.g. nictitating membrane and rhythmic breathing) would suggest this may not be the case, and the presence of jaw tone may be indicative of damage to the larynx (Cors *et al* 2015; Silvano *et al* 1996), which can result in spontaneous “gagging” or perceived “gasping” behaviours and resulting in perceived jaw tone. These behaviours are not indicative of consciousness and are present in the absence of auditory evoked potentials (Cors *et al* 2015).

The ceasing of clonic death-related behaviours (e.g. leg paddling and wing flapping) has been used as an indicator of time of death for poultry which are killed by CO_2 gas stunning (Gerritzen *et al* 2007), and based on this, all three killing methods were shown to kill birds in similar time periods, despite small differences attributed to bird type and age, which may be indicative of variation in bird nutrition and available muscle glycogen (Debut *et al* 2015; Petracci *et al* 2010). The majority of birds showed convulsive wing flapping and leg paddling, which has been observed in several other studies of killing with various methods (Abeyesinghe *et al* 2007; Lambooij *et al* 1999; McKeegan *et al* 2007). The onset of cloacal movement, where visible, was the last reflex observed before all movements ceased, which may highlight it as a conservative indicator of death.

Conclusion and Animal Welfare Implications

The NMCD was effective at killing layers and broilers of various ages and weights reliably and causing loss of reflexes within a short period of time. The NMCD maintained the kill success of MCD, but improved the technique and consistency of its application. After application of NMCD, birds were likely to become unconscious rapidly due to extensive trauma to the brain stem and/or spinal cord (highlighted by immediate loss of reflexes in the majority of birds which indicate consciousness) and die from cerebral ischemia due to severing of carotid arteries. The MZIN device had a kill success rate of only 72%, making it unsuitable for use despite rapid loss of reflexes when it was successful. Only NMCD and MCD can be considered to be the most humane of the three methods tested here due to their 100% success rate and inducement of rapid reflex loss; indeed a high proportion of birds never showed reflexes at all post-application.

Collectively, these results suggest that NMCD is the most promising device in terms of kill success rate (reliability), humaneness and consistency of the methods tested here.

References

Abeyesinghe S M, McKeegan D E F, McLeman M A, Lowe J C, Demmers T G M, White R P, Kranen R W, Van Bemmel H, Lankhaar J A C and Wathes C M 2007 Controlled atmosphere stunning of broiler chickens. I. Effects on behaviour, physiology and meat quality in a pilot scale system at a processing plant. *British Poultry Science 48:* 406-423

Accles and Shelvoke 2010 Cash Poultry Killer Model CPK200 Product Data Sheet. Accles & Shelvoke : UK

Anil M H 1991 Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Science 30:* 13-21

Aslan K, Atalgin H, Kurtul I and Bozkurt E U 2006 Patterns of the internal and cerebral carotid arteries in avrious avian species: a comparative study. *Revue Med Vet 157:* 621-624

Aviagen 2009 Ross 308 Broiler: Management Manual.

AVMA 2007 AVMA guidelines on euthanasia. pp 669-696. AVMA: United States

Bader S, Meyer-Kûhling B, Günther R, Breithaupt A, Rautenschlein S and Gruber A D 2014 Anatomical and histologic pathology induced by cervical dislocation following blunt head trauma for on-farm euthanasia of poultry. *Journal of Applied Poultry Research 23:* 546-556

Bilello J F, Davis J W, Cunningham M A, Groom T F, Lemaster D and Sue L P 2003 Cervical Spinal Cord Injury and the Need for Cardiovascular Intervention. *Arch Surg 138:* 1127-1129

Blackman N L, Cheetham K and Blackmore D K 1986 Differences in blood supply to the cerebral cortex between sheep and calves during slaughter. *Research in Veterinary Science 40:* 252-254

Blackmore D K and Delany M W 1988 Slaughter of Stock. A Practical Review and Guide. pp 1-134. Veterinary Continuing Education, Massey University: New Zealand

Brieg A 1970 Overstretching of and Circumscribed Pathological Tension in the Spinal Cord - A Basic Cause of Symptoms in Cord Disorders. *J.Biomechanics 3:* 7-9

Carbone L G, Carbone E T, Yi E M, Bauer D B, Lindstrom K A, Parker J M, Austin J A, Seo Y, Gandhi A D and Wilkerson J D 2012 Assessing cervical dislocation as a humane euthanasia method for mice. *Journal of the American Association for Laboratory Animal Science 51:* 352-356

Cartner S C, Barlow S C and Ness T J 2007 Loss of Cortical Function in Mice After Decapitation, Cervical Dislocation, Potassium Chloride Injection, and CO2 Inhalation. *Comparative Medicine 57:* 570-573

Claassen J, Carhuapoma J R, Kreiter K T, Du E Y, Connolly E S and Mayer S A 2002 Global cerebral edema after subarchnoid hemorrhage: frequency, predictors, and impact on outcome. *Stroke 33:* 1225-1232

Close B, Banister K, Baumans V, Bernoth E, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N G, Hackbarth H, Morton D and Warwick C 2007 Recommendations for euthanasia of experimental animals: Part 2. *Laboratory Animals 31:* 1-32

Cors J C, Gruber, A D, Günther R, Leyer-Kühling B, Esser K H, and Rautenschlein S 2015 Electroencephalographic evaluatin of the effectiveness of blunt trama to induce loss of consciousness for on-farm killing of chickens and turkeys. *Poultry Science* 00: 1-9

Croft P G 1961 The photomotor reflex as an indicator of consciousness in the immobolized dog. *Journal of Small Animal Practise 2:* 206-214

Dawson M D, Johnson K J, Benson E R, Alphin R L, Seta S and Malone G W 2009 Determining cessation of brain activity during depopulation or euthanasia of broilers using accelerometers. *Journal of Applied Poultry Research 18:* 135-142

Dawson M D, Lombardi M E, Benson E R, Alphin R L and Malone G W 2007 Using accelerometers to determine the cessation of activity in broilers. *Journal of Applied Poultry Research 16:* 583-591

Day S, Cook E F, Funkenstein H and Goldman L 1982 Evaluation and outcome of emergency room patients with transient loss of consciousness. *The American Journal of Medicine 73:* 15-23

Debut M, Berri C, Arnould C, Guemené D, Santé-Lhoutellier V, Sellier N, Baéza E, Jehl N, Jégo Y, Beaumont C, and Le Bihan-Duval E 2015 Behavioural and physiological responses of three chicken breeds to pre-slaughter shackling and acute head stree. *British Poultry Science* 46(5):527-535

DEFRA 2002a Code of recommendations for the welfare of livestock: laying hens. Defra Publications: London

DEFRA 2002b Codes of recommendations for the welfare of livestock:Meat chickens and breeding chickens. Defra Publications: London

DEFRA 2014 DEFRA (MH0145) Welfare costs and benefits of exizting and novel on-farm culling methods for poultry. In:McKeegan, D E F, Martin, J E, Sandilands, V, Sandercock, D A, Sparrey, J M and Sparks, N H C (eds) DEFRA Publications: UK

Drew L B and Drew W E 2004 The Contrecoup-Coup Phenomenon. *Neurocritical Care 1:* 385-390

Dumont R J, Okonkwo D O, Verma S, Hurlbert R J, Boulos P T, Ellegala D B and Dumont A S 2001a Acute Spinal Cord Injury, Part I: Pathophysiologic Mechanisms. *Clinical Neuropharmacology 24:* 254-264

Dumont R J, Verma S, Okonkwo D O, Hurlbert R J, Boulos P T, Ellegala D B and Dumont A S 2001b Acute Spinal Cord Injury, Part II: COntemporary Pharmacotherapy. *Clinical Neuropharmacology 24:* 265-279

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

Erasmus M A, Turner P V, Nykamp S G and Widowski T M 2010b Brain and skull lesions resulting from use of percussive bolt, cervical dislocation by stretching, cervical dislocation by crushing and blunt trauma in turkeys. *Veterinary Record 167:* 850-858

Erasmus M A, Turner P V and Widowski T M 2010c Measures of insensibility used to determine effective stunning and killing of poultry. *Journal of Applied Poultry Research 19:* 288-298

European Council 2009 European Council Regulation (EC) 1099/2009 of 24 September 2009 on the protection of animals at the time of killing.

Freeman L W and Wright T W 1953 Experimental Observations of Concussion and Contusions of the Spinal Cord. *Annals of Surgery 137:* 433-443

Gerritzen M A, Lambooij H, Reimert H, Stegeman A and Spruijt B 2007 A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. *Applied Animal Behaviour Science 108:* 179-185

Grandin T 1994 Public veterinary medicine: food safety and handling. Euthanasia and slaughter of livestock. *Journal of American Veterinary Medicine Association 212:* 39

Gregory N G 1991 Humane Slaughter. *Outlook on Agrciulture 20:* 95-101

Gregory N G, Schuster P, Mirabito L, Kolesar R and McManus T 2012 Arrested blood flow during false aneurysm formation in the carotid arteries of calttle slaughtered with and without stunning. *Meat Science 90:* 368-372

Gregory N G and Wotton S B 1986 Effect of slaughter on the spantaneous and evoked activity of the brain. *British Poultry Science 27:* 195-205

Gregory N G and Wotton S B 1990 Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. *Veterinary Record 126:* 570-572

Harrop J, Sharan A, Vaccaro A R and Przybylski G J 2001 The Cause of Neurologic Deterioration After Acute Cervical Spinal Cord Injury. *Spine 26:* 340-346

Heard D 2000 Perioperative supportive care and monitoring. *Vet.Clin.North Am.Exot.Anim.Pract. 3:* 587-615

Hellyer P W, Freeman L C and Hubbell J A E 1991 Induction of Anesthesia with Diazepam-Ketamine and Midazolam-Ketamine in Greyhounds. *Veterinary Surgery 20:* 143-147

Hogg D A 1982 Fusions occurring in the postcranial skeleton of the domestic fowl. *J Anat 135:* 501-512

HSA 2004 Practical Slaughter of Poultry: A Guide for the Small Producer. Humane Slaughter Association:

Hy-Line 2012 Hy-Line Brown Performance Standards Manual. *2th edition*

Krause G S, White B C and Aust S D 1988 Brain cell death after ischemia and reperfusion: A proposed biochemical sequence. *Crit Care Med 16:* 714-726

Kushner D 1998 Mild traumatic brain injury. *Arch Intern Med 158:* 1617-1624

Lambooij E, Gerritzen M A, Engel B, Hillebrand S J W, Lankhaar J and Pieterse C 1999 Behavioural responses during exposure of broiler chickens to different gas mixtures. *Applied Animal Behaviour Science 62:* 255-265

LeBlang S D and Nunez D B 2000a Noninvasive imaging of cervical vascular injuries. *AJR 174:* 1269-1278

LeBlang S D and Nunez D B 2000b Noninvasive inaging of cervical vascular injuries. *American Journal of Roentgenology 174:* 1269-1278

Martin J E 2015 Humane mechanical methods to kill poultry on-farm. *PhD Thesis* University of Glasgow, UK

Martin P and Bateson P 2007 Measuring Behaviour. An Introductory Guide. *Cambridge University Press* 92

Mason C, Spence J, Bilbe L, Hughes T and Kirkwood J 2009 Methods for dispatching backyard poultry. *Veterinary Record 164:* 220

McKeegan D E F, Abeyesinghe S M, McLeman M A, Lowe J C, Demmers T G M, Whiting T L, Kranen R W, Van Bemmel H, Lankhaar J A C and Wathes C M 2007 Controlled atmsphere stunning of broiler chickens. II. Effects on behvaiour, physiology and meat quality in a commercial processing plant. *British Poultry Science 48:* 430-442

McKeegan D E F, Reimert H G M, Hindle V A, Boulcott P, Sparrey J M and Gerritzen M A 2013 Physiological and behavioural responses of poultry exposed to gas filled high expansion foam. *Poultry Science 92:* 1145-1154

McLeod W M, Trotter D M and Lumb J W 1964 Avian Anatomy. pp 19-20. Burgess Publishing Company: United States of America

Ommaya A K and Gennarelli T A 1974 Cerebral concussion and traumatic unconsciousness. *Brain 97:* 633-654

Parent A, Harkey L H, Touchstone D A, Smit E E and Smith R R 1992 Lateral Cervical Spine Dislocation and Vertebral Artery Injury. *Neurosurgery 31:* 501-509

Petracci M, Bianchi M, and Cavani C 2010 Pre-slaughter handling and slaughtering factors infleuncing poultry product quality. *World's Poultry Science Journal* 66(1): 17-26

Perry M O, Snyder W H and Thal E R 2012 Carotid artery injuries caused by blunt force trauma. *Carotid Artery Injuries 192:* 74-77

Pizzurro S 2009a About us - expectation of order fulfilment.

Pizzurro S 2009b Zinger Stun Guns™ - The Rabbit Zinger™, (TRZ001).

Pryor J D and Shi R 2006 Electrophysiological changes in isolated spinal cord white matter in response to oxygen deprivation. *Spinal Cord 44:* 653-661

Raj A B M, O'Callaghan M and Knowles T G 2006 The effects of amount and frequency of alternating current used in water bath stunning and of slaughter methods on electroencephalograms in broilers. *Animal Welfare 15:* 7-18

Sandercock D A, Auckburally A, Flaherty D, Sandilands V and McKeegan D E F 2014 Avian reflex and electroencephalogram responses in different states of consciousness. *Physiology & Behavior 133:* 252-259

Sharma B R, Singh V P and Harish D 2005 Neck Structure Injuries in Hanging - Comparing Retrospective and Prospective Studies. *Med.Sci.Law 45:* 321-330

Shaw N A 2002 The neurophysiology of concussion. *Progress in Neurobiology 67:* 344

Shi R and Pryor J D 2002 Pathological Changes of Isolated Spinal Cord Axons in Respnse to Mechanical Stretch. *Neuroscience 110:* 765-777

Shi R and Whitebone J 2006 Conduction Deficits and Membrane Disruption of Spinal Cord Axons as a Function of Magnitude and Rate of Strain. *J Neurophysiol 95:* 3384-3390

Silvano C, Gemma M, De Vitis A, Piccoli S, Frascoli C, and Beretta L 1996 Difficult Diagnosis of Laryngeal Blunt Trauma. Journal of Trauma-Injury Infection & Critical Care 40(5): 845-846

Solomon 1990 Human Anatomy and Physiology. pp 400-539. Saunders College Publishing:

Sparrey J M, Sandercock D M, Sparks N H C and Sandilands V 2014 Current and novel methods for killing poultry individually on-farm. *World's Poultry Science Journal* 70(4) 737-758

Takahashi H, Manaka S and Sano K 1981 Changes of extracelluar potassium concentration in the cortex and brain stem during acute phase of experimental closed head injury. *No To Shinkei 33:* 365-376

Tidswell S J, Blackmore D K and Newhook J C 1987 Slaughter methods: Electroencephalographs (EEG) studies on spinal cord section, decaptitation and gross trauma of the brain in lambs. *New Zealand Veterinary Journal 35:* 46-49

Veras L, Pedraza-Gutiérrez S, Castellanos J, Capellades J, Casamitjana J and Rovira-Cañellas A 2000 Vertebral Artery Occlusiion After Acute Cervical Spine Trauma. *Spine 25:* 1171-1177

Weir C J, Zivin J A and Lyden P D 2002 Inter-relationships between spinal cord blood flow, neuronal death and neurological function in rabbit spinal cord ischemia. *Brain Research 946:* 43-51

White B C and Krause G S 1993 Brain injury and repair mechanisms: the potential for pharmacologic therapy in closed-head trauma. *Annals of Emergency Medicine 22:* 970-979

Whittow G C 2000 Sturkie's Avian Physiology. pp 71-80. Academic Press: UK

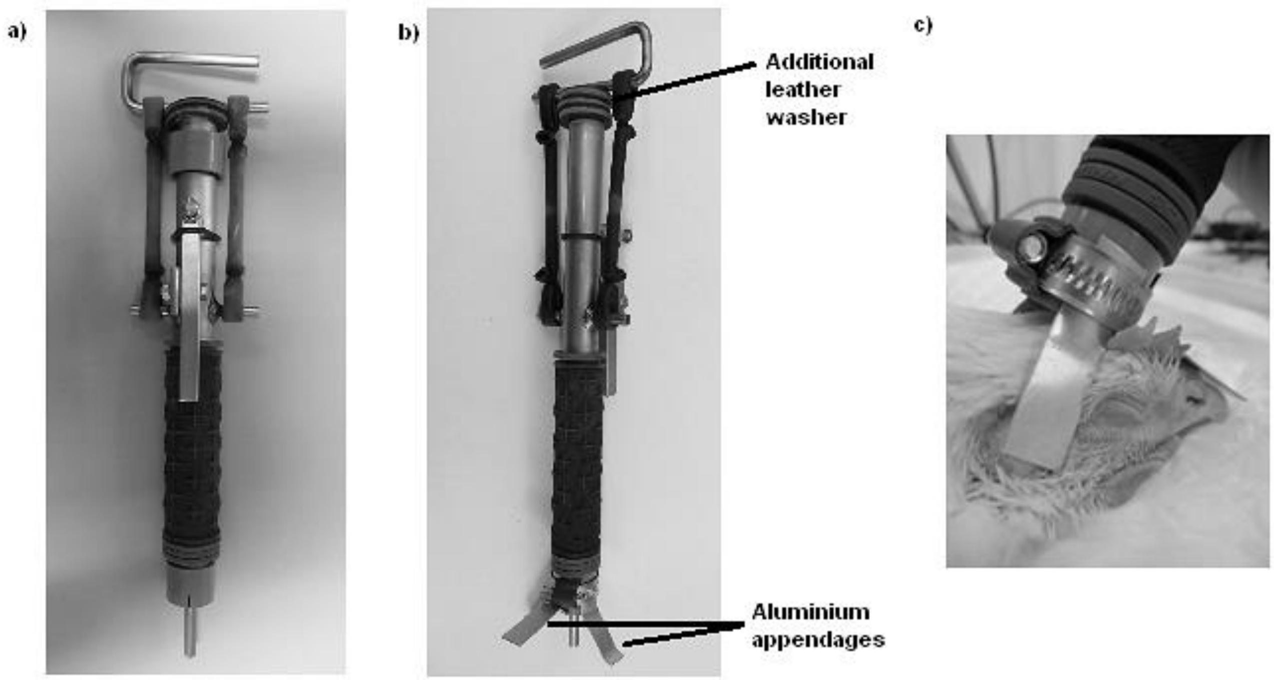

Figure 1: Photographs of (a) the original Rabbit Zinger^{TM}; (b) the modified Rabbit Zinger (MZIN) in un-cocked states; and (c) demonstration of MZIN in position on a cadaver. Both are approximately 35 cm in length (un-cocked) and approximately 50 cm (cocked).

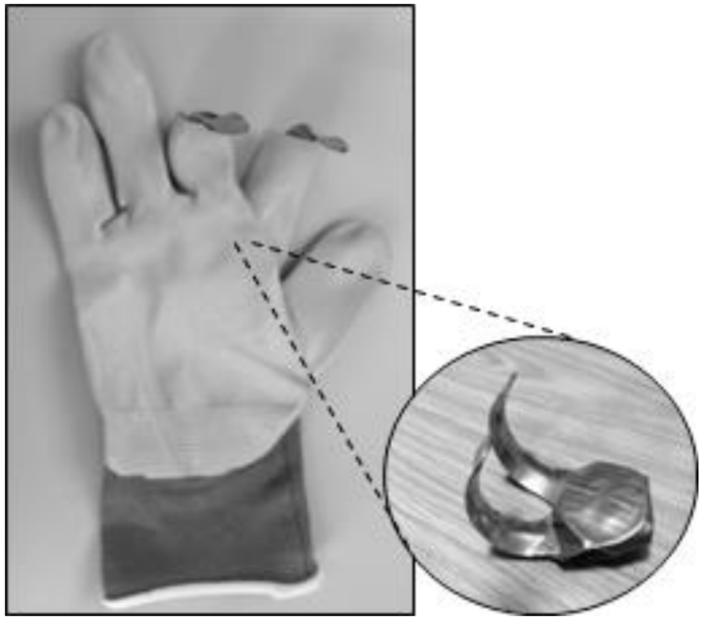

Figure 2: The completed NMCD device: metal inserts in situ within the glove. The metal inserts were secured with Velcro® (Velcro VEL-EC60214 20mm x 2.5m Brand Stick on Tape, Velcro Industries, UK) within the glove. The metal insert was produced with the aid of an engineer (Julian Sparrey). The device was designed to be tight fitting in order to maintain relatively strong tactile sensation for the operator through the glove, in order to correctly adjust the metal fingers where necessary.

a) b)

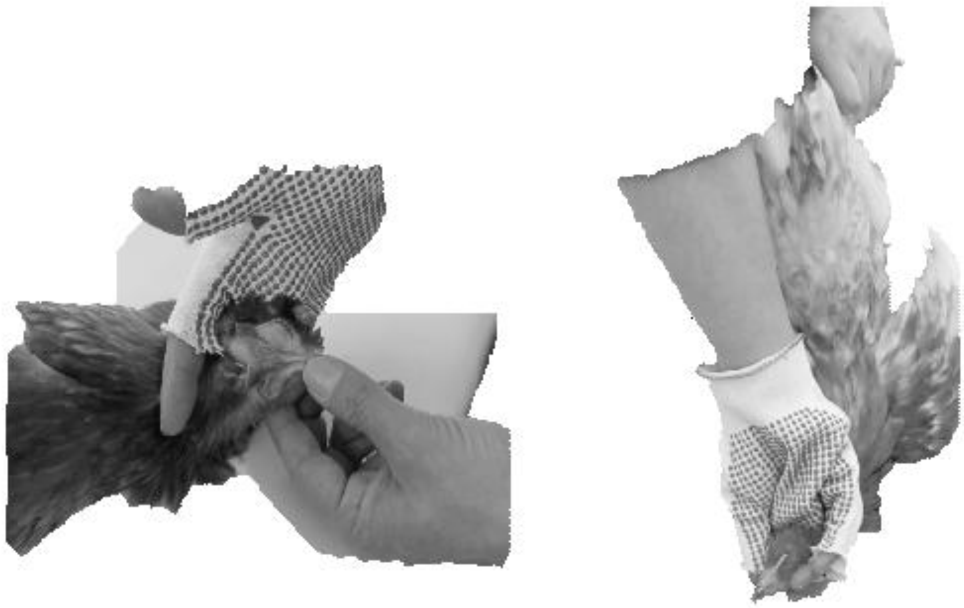

Figure 3: Demonstrating how the NMCD device is: (a) placed around the bird's head; and (b) applied to a bird. The operator's index and middle fingers rested above the finger inserts and the hinge joint sat on the fleshy pad below the fingers. The tips of the metal fingers rested under the bird's jaw and the metal hinge joint rested behind the bird's skull, at the top of the neck. The operator secured the bird's head in place by placing their thumb and ring finger under the bird's chin. The operator's un-gloved hand was used to hold the bird's legs (securing the bird upside down), resting its underside against the operator's thigh. The device was applied in one swift movement with the gloved hand pulling downwards on the head, while also rotating the head back towards the ceiling and forcing the metal edge into the back of the bird's head and the top of the neck.

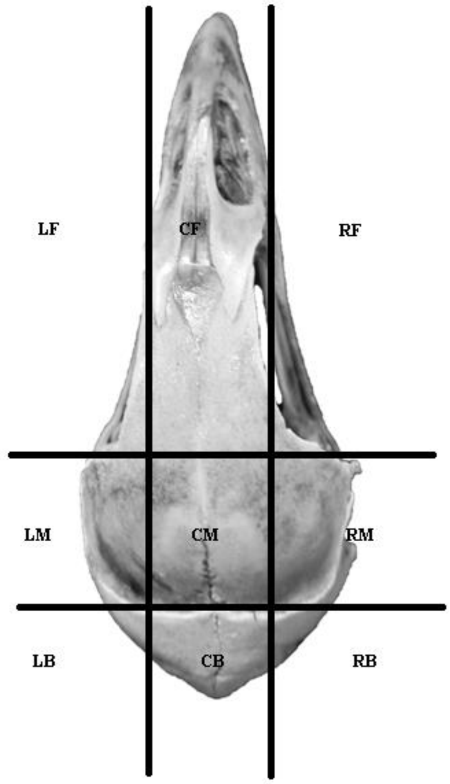

Figure 4: Photograph of a layer hen skull (38 weeks old) indicating the nine skull penetration areas mapped during post mortem examinations; areas are separated into 3 regions: left (L), centre (C) and right (R) and then split into the Front (F), Mid (M), and Back (B).

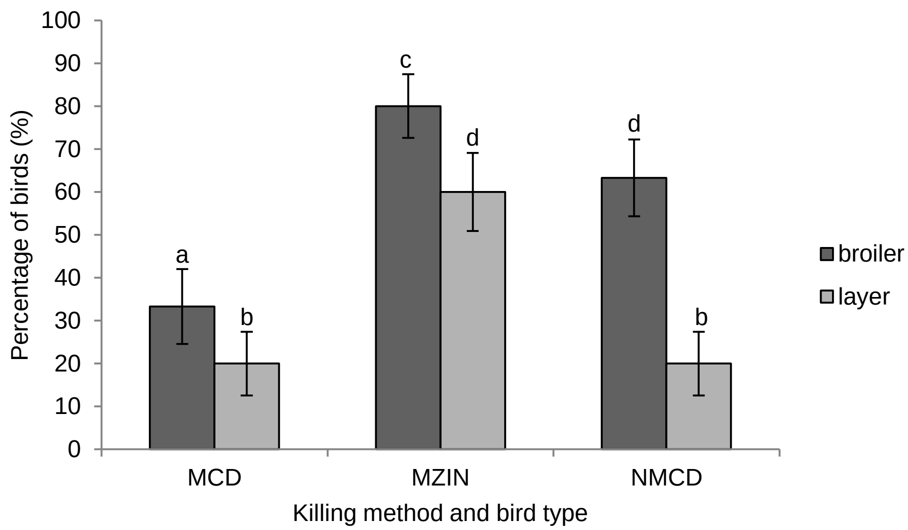

Figure 5: Mean (± SE) device success rates (%) across the three killing methods and bird type (broiler/layer).

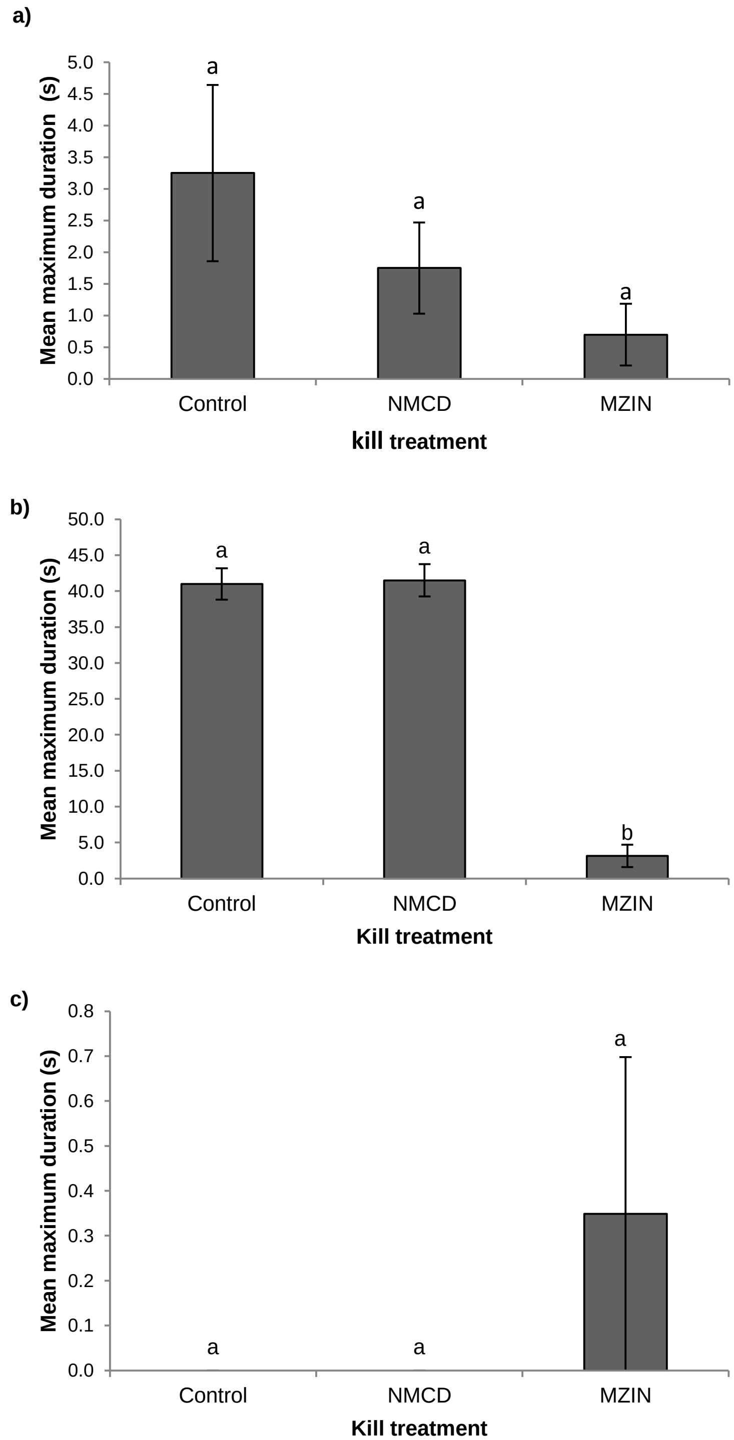

Figure 6: Mean (±SE) of the maximum durations (s) across the three killing treatments for the cranial reflexes: a) nictitating membrane ($F_{2,150}$ = 1.67, P = 0.191); b) pupillary ($F_{2,150}$ = 101.66, P < 0.001); and c) rhythmic breathing ($F_{2,150}$ = 1.46, P = 0.235). Note that y axes ranges differ.

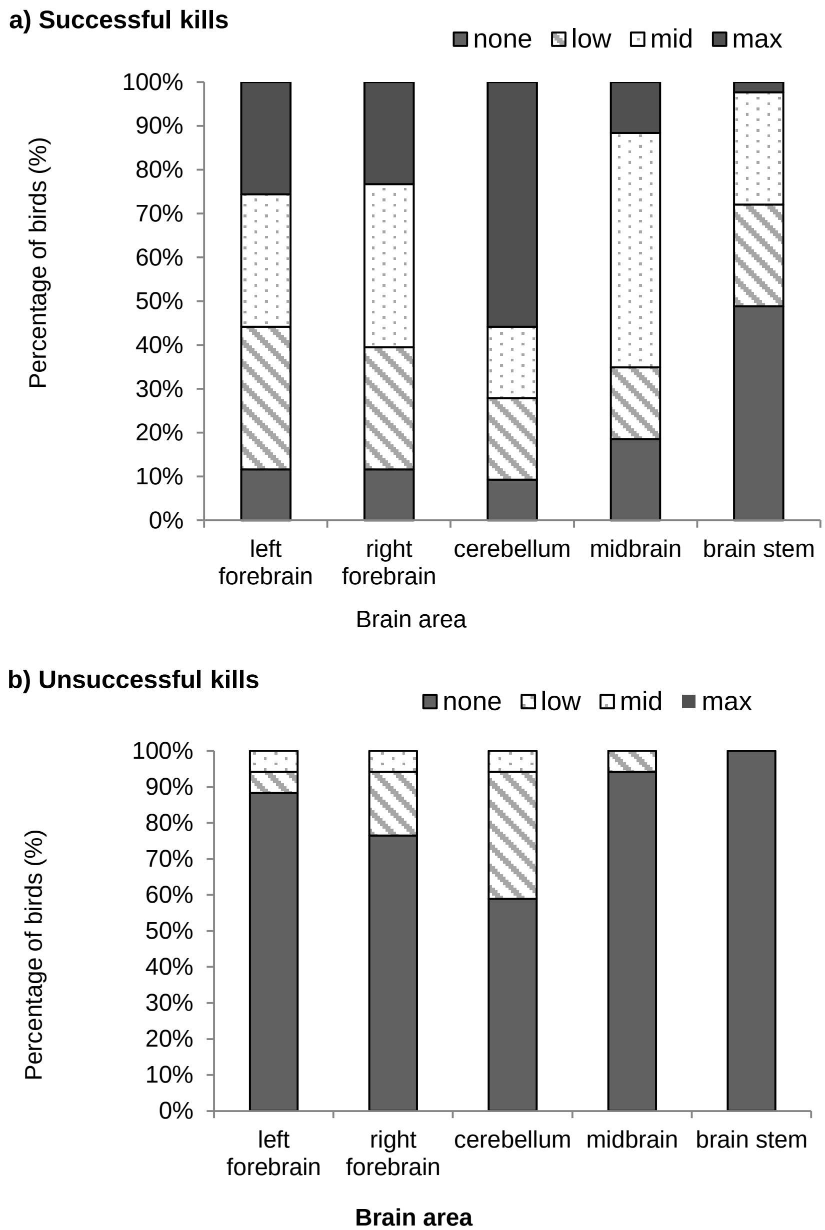

Figure 7: Comparison of brain damage ranges for successful and unsuccessful kills by the MZIN. Refer to Table 3 for defined damage grading.

Table 1: Accommodation and bird details for each bird type and age group

Bird group	Age	Mean weight (kg)	N per pen	Pen furniture
Layer pullets (Hy-Line strain)	Batch 1 – 10 wks Batch 2 – 13 wks	1.08 ± 0.02	3-4	1 feeder, 3 automatic cup drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string. Total of 6 pens.
Layer hens (Hy-Line strain)	Batch 1 – 58 wks Batch 2 – 63 wks	1.79 ± 0.03	3-4	1 feeder, 3 automatic cup drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string. Total of 6 pens.
Broiler chicks (Ross 308 strain)	Batch 1 – 3 wks Batch 2 – 2 wks	0.71 ±0.02	22-23	2 x feeder, 1 x automatic large bell drinker, 4 x suspended shiny objects. One large pen housed all chicks.
Broiler – slaughter age (Ross 308 strain)	Batch 1 – 5 wks Batch 2 – 5 wks	2.17 ± 0.06	2-3	1 feeder, 3 automatic cup drinkers, 2 x suspended shiny objects. Total of 10 pens.

Table 2: List of reflexes and involuntary behaviours, recorded in order of observation after application of -killing treatment, with the specific cranial nerve pathway and identified brain area for control (reference) as well as the procedure used to assess them as present or absent.

Reflex/ Behaviour	**Code**	**Neurological control area**	**Procedure**
Pupillary (light) reflex	PUP	Cranial nerve II/III (Midbrain)	Constriction reaction of the pupil to light directed into the eye from a medical pen light approximately 5cm from the corneal surface
Nictitating membrane reflex	NIC	Cranial nerve V/IV (Midbrain)	In response to mechanical touch stimulation (via pressing of a probe) of the medial canthus, the nictitating membrane (palpebra tertia) transiently closes over the surface of the eye
Rhythmic breathing	RB	Cranial nerve X (Brain stem)	Observations of >3 consecutive breaths from visual confirmation of the rib cage moving up and down rhythmically.
Jaw tone	JT	Cranial nerve IV (Brain stem)	Resistance observed due to downward manipulation and pressure applied to the lower beak
Cloacal movement	VW	Cranial nerve X (Brain stem)	Visual observation of sporadic opening and closing of the cloaca in a “puckering” movement
Wing flapping	WF	Spinal cord effectors (Brain stem)	Observation of clonic flapping of the wings in a sporadic fashion
Leg paddling	LP	Spinal cord effectors (Brain stem)	Observation of clonic movement of the legs in a sporadic fashion

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Table 3: Grading system for categorising levels of damage to individual areas of the brain for treatment MZIN

Damage grading	Description
None	No damage to the specific region of the brain, no visible bruising or physical damage.
Low	Region of brain is physically intact; however there is visible bruising and pooling of blood in the surrounding area.
Mid	Region of brain shows visible signs of physical damage, but is still in-situ. There is visual bruising and bleeding in the surrounding area.
Max	Region of brain shows extensive physical damage, with some or all parts no longer in-situ. There is visually obvious bruising and bleeding in the surrounding area.

Table 4: GLMM analysis results for mean maximum durations (s) for jaw tone, leg paddling, wing flapping and cloacal movement post kill treatment application, in successfully killed birds only (N = 163).

		Wing flapping		Leg paddling		Cloacal movement		Jaw tone	
Fixed Effects	*df*	*F statistic*	*P*	*F statistic*	*P*	*F statistic*	*P*	*F statistic*	*P*
Killing treatment	2,150	2.05	0.132	3.18	0.044	3.75	0.026	13.34	<0.001
Bird type	1,150	41.71	<0.001	35.35	<0.001	18.32	<0.001	2.46	0.119
Bird age	1,150	6.83	0.010	8.02	0.005	21.45	<0.001	0.34	0.563
Bird weight	1,150	2.57	0.111	2.18	0.142	4.47	0.036	2.48	0.117
Device success	1,150	0.93	0.337	0.33	0.565	0.11	0.744	1.28	0.260
Treatment.bird type	2,150	1.16	0.315	0.57	0.567	1.65	0.196	3.73	0.026
Treatment.bird age	2,150	2.23	0.111	2.23	0.111	0.63	0.533	4.58	0.012
Bird age.bird weight	2,150	1.81	0.168	2.21	0.113	0.57	0.568	3.99	0.020

Table 5: Mean maximum durations (s) to loss of relevant behaviours for significant factors bird type and bird age. Means with different superscript letters indicate that there was a significant difference P<0.05.

	Jaw tone		Wing flapping		Leg paddling		Cloacal movement	
Factor	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Broiler	5.3^{a}	0.9	82.5^{a}	4.6	84.2^{a}	5.0	94.3^{a}	4.8
Layer	8.2^{a}	1.1	113.5^{b}	6.1	113.5^{b}	6.0	113.5^{b}	6.9
Young (early production stage)	4.6^{a}	1.8	77.0^{a}	7.1	76.3^{a}	7.8	90.0^{a}	7.2
Old (late production stage)	6.5^{a}	2.1	110.5^{b}	11.1	113.2^{b}	11.3	119.3^{b}	12.4

Table 6: Percentage of successfully-killed birds which displayed the reflexes and involuntary behaviours for each killing treatment.

Reflex/ behaviour	Control (N = 60)	NMCD (N = 60)	MZIN (N = 43)
Pupillary	100.0	98.3	11.6
Nictitating membrane	10.0	10.0	3.3
Rhythmic breathing	0.0	0.0	1.7
Jaw tone	28.3	38.3	21.7
Cloacal movement	95.0	95.0	98.3
Wing flapping	100.0	100.0	98.3
Leg paddling	100.0	100.0	98.3

Table 7: The percentage of birds which exhibited tissue damage across the range of post-mortem measures, related to kill success for the MZIN.

	Percentage of birds observed in (%)		
Post-mortem measure	Kill Success 'Yes'	Kill Success 'No'	*P* value
Skin broken	95.4	88.2	0.360
External bleeding	95.4	88.2	0.360
Subcutaneous hematoma[+]	100.0	100.0	-
Skull damage	100.0	58.8	0.024
Internal brain cavity hematoma	100.0	64.7	0.018
Left forebrain damage	88.4	11.8	<0.001
Right forebrain damage	88.4	23.5	<0.001
Cerebellum damage	90.7	41.2	0.028
Midbrain damage	81.4	5.9	<0.001
Brain stem damage	51.2	0.0	<0.001

[+]GLMM not calculated as both 100 % for kill success fixed effect