

Scotland's Rural College

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

Martin, JE; McKeegan, DEF; Sparrey, J; Sandilands, V

Published in:
Animal Welfare

DOI:
[10.7120/09627286.25.2.227](https://doi.org/10.7120/09627286.25.2.227)

First published: 01/05/2016

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):

Martin, JE., McKeegan, DEF., 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. *Animal Welfare*, 25(2), 227 - 241. Advance online publication. <https://doi.org/10.7120/09627286.25.2.227>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

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

3 Jessica E Martin^{1,2*}, Dorothy E F McKeegan², Julian Sparrey³ and Victoria Sandilands¹

4

5 ¹ Avian Science Research Centre, Animal and Veterinary Sciences Group, SRUC Auchincruive, Ayr, KA6 5HW,
6 UK

7 ² Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary & Life
8 Sciences, University of Glasgow, G61 1QH, UK

9 ³ Livetec Systems Ltd., Building 52 Wrest Park, Silsoe, Bedford, Bedfordshire, MK45 4HS, UK

10

11 *Contact for correspondence: Jessica.Martin@sruc.ac.uk; Animal Behaviour and Welfare, Animal and Veterinary
12 Science Research Group, SRUC, Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK

13

14 **Abstract**

15 An alternative emergency method for killing poultry on-farm is required following European
16 legislation changes (EU 1099/2009), which heavily restricts the use of manual cervical
17 dislocation. This study investigated the kill efficacy of two mechanical methods that conform to
18 the new legislation: (1) a novel mechanical cervical dislocation device; and (2) a modified
19 captive bolt device (Rabbit ZingerTM) and manual cervical dislocation (the control). Killing
20 treatments were applied to broilers and layers at two stages of production (broilers: 2-3 weeks
21 and 5 weeks of age; layers: 12-13 weeks and 58-62 weeks), with a total of 180 birds. Latency to
22 abolition of cranial and behavioural reflexes, as well as post-mortem analysis of the

23 physiological damage produced, were used to estimate time to unconsciousness and assess kill
24 efficacy. The novel mechanical cervical dislocation device was reliable and a practical method
25 for killing poultry on-farm, with 100% kill success ($F_{2,167} = 19.96, P < 0.001$) and cranial reflex
26 interval mean durations lasting for 0.0 - 103.0 s post-kill (jaw tone ($F_{2,150} = 13.34, P < 0.001$);
27 pupillary ($F_{2,150} = 101.66, P < 0.001$); nictitating membrane ($F_{2,150} = 1.61, P = 0.191$); and
28 rhythmic breathing ($F_{2,150} = 1.46, P = 0.235$) compared to the modified Rabbit ZingerTM (72% kill
29 success rate, 0.3-124.9 s cranial reflex mean durations) and manual cervical dislocation (100%
30 kill success rate, 0.0-119.3 s cranial reflex mean durations). The novel mechanical cervical
31 dislocation device resulted in consistent anatomical damage to the birds (e.g. high dislocation of
32 the neck and severing of the spinal cord) compared to the manual method, despite both having
33 100% success rate, while the modified Rabbit ZingerTM was difficult to operate and resulted in
34 varied anatomical damage. The novel mechanical cervical dislocation device showed promise as
35 a replacement kill method on farm for poultry.

36

37 **Keywords**

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

39

40 **Introduction**

41 Determining the efficacy of on-farm killing methods for individual birds is essential to poultry
42 welfare in both commercial and non-commercial contexts. Poultry may need to be killed on farm
43 or in backyard flocks for several reasons (e.g. in an emergency for small-scale disease control or

44 injury, and for stock management). Emergency killing of large numbers of birds are often
45 controlled by whole-house or containerised gas methods, or birds may be transported for
46 slaughter and then slaughtered using gas or electrical waterbath stunning methods. However, for
47 individual birds on-farm, there are two key methods for killing poultry: (1) cervical dislocation,
48 which is designed to cause death by cerebral ischemia and extensive damage to the spinal cord
49 and brain stem (Bader *et al* 2014; Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory & Wotton
50 1990; Ommaya & Gennarelli 1974); and (2) percussive devices designed to cause extensive
51 brain damage, resulting in brain death (Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory &
52 Wotton 1990; HSA 2004; Mason *et al* 2009; Sparrey *et al* 2014).

53

54 Cervical dislocation methods can be divided into two categories: (1) manual – cervical
55 dislocation of the neck by hand (MCD); and (2) mechanical – cervical dislocation of the neck
56 with the aid of a tool (Gregory & Wotton 1990; HSA 2004; Mason *et al* 2009; Sparrey *et al*
57 2014). The most common method for despatching poultry on-farm is manual cervical dislocation
58 (MCD) (Mason *et al* 2009), as it is perceived to be humane by users, easy to learn and perform,
59 and does not require equipment. All cervical dislocation killing methods are designed to separate
60 the skull from the vertebral column of the bird (C0-C1 vertebral dislocation), resulting in
61 severing of the spinal cord and/or brain stem and the main blood vessels supplying the brain
62 (Cartner *et al* 2007; Gregory & Wotton 1990; Mason *et al* 2009; Parent *et al* 1992; Veras *et al*
63 2000). It has been suggested that optimal application also produces a concussive effect on the
64 bird due to trauma inflicted on the brain stem through the action of stretching and twisting
65 (Cartner *et al* 2007; Erasmus *et al* 2010a; Harrop *et al* 2001; Pryor & Shi 2006; Shi & Pryor
66 2002; Shi & Whitebone 2006). However, both methods of cervical dislocation (but MCD in

67 particular, perhaps because it is more common) have been the subject of welfare concern, as
68 research in the last 40 years has questioned their humaneness and consistency in poultry
69 (Erasmus *et al* 2010a; Gregory & Wotton 1986; Gregory & Wotton 1990), as well as other
70 species (Cartner *et al* 2007; Tidswell *et al* 1987). Some studies have indicated that animals,
71 including poultry, may be conscious for a significant period post-application of cervical
72 dislocation methods (Carbone *et al* 2012; Erasmus *et al* 2010a; Gregory & Wotton 1990) and it
73 has been noted that there is high variability in its application across different relevant groups
74 (e.g. poultry stock-workers, veterinarians, trained slaughtermen) (Mason *et al* 2009; Sparrey *et al*
75 2014). In response to these concerns, as of January 2013, the use of MCD has been restricted
76 through European legislation (EC 1099/2009) to a maximum of 70 birds/person/day and to birds
77 ≤ 3 kg in weight (European Council 2009). As a result, an alternative method for killing poultry
78 on-farm needs to be identified which conforms to the new legislation and is proven to be
79 effective and humane.

80

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

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

94

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

99

100 **Materials and Methods**

101 *Animal housing and husbandry*

102 A total of 180 female chickens were used for the study. The birds were tested in two batches of
103 90 birds on separate days. In each batch 15 layers and 15 broilers, each divided into two age
104 classes (either 7 or 8 birds per type/age-class depending on the day tested, but always totalling 15
105 over the two days), were assessed for each of the three killing treatments (N=15). Further details
106 about the birds and their accommodation are provided in Table 1. The sample size was chosen to
107 allow significant differences to be identified in behavioural data which is prone to high
108 individual variation across two bird types and two bird age groups (within type) across three
109 killing treatments. A minimum of 12 birds were calculated to provide sufficient power in the

110 analysis (88%), however an additional 3 birds were used per treatment group in order to
111 compensate for any unsuccessful birds and therefore the loss of valid behavioural data.

112

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

123

124 *Table 1*

125

126 ***Study design***

127 Two novel mechanical poultry killing devices, the Modified Rabbit Zinger (MZIN) and a novel
128 mechanical cervical dislocation gloved device (NMCD), were assessed for their kill efficacy in
129 comparison with each other and a control (MCD). The Rabbit ZingerTM (Pizzurro 2009a;
130 Pizzurro 2009b) is a penetrating captive bolt device originally designed to kill rabbits that uses
131 the stored energy in rubber tubes to drive a penetrating bolt into the head, causing death by

132 extensive irreversible brain damage (DEFRA 2014; Martin 2015) (Figure 1a). The device was
133 modified with permission of the original designer in order to adapt it to the new target species
134 (Figure 1b), however the original function and bolt mechanism of the device was retained. The
135 blue Power Tubes™ (Pizzurro 2009a) were used, which require 177 N to pull the bolt into the
136 cocked position (Sparrey *et al* 2014) and when fired the bolt delivered approximately 11.87 J of
137 kinetic energy. The modifications consisted of three aluminium appendages added to the base of
138 the device in order to secure the bird's head in place between them: two rested either side of the
139 bird's head (over the ears, or auricular feathers) and the third ran down the front of the bird's
140 face between the eyes and over the nostrils and beak (Figure 1c). The appendages were designed
141 to position the bird's head correctly in order to direct the bolt (0.6 mm diameter) into the bird's
142 brain and brain stem. Additional leather washers were added to the bolt, in order to reduce the
143 penetration depth from approximately 3.5 cm to 2.5 cm. The device was also weighted at the
144 bottom in order to counteract the top heaviness of the device when cocked.

145

146 *Figure 1*

147

148 The NMCD device (Figure 2) was designed to create a mechanical method for cervical
149 dislocation of poultry which mirrored the technique of the manual method. The device consisted
150 of a supportive glove (SHOWA 370 Multi-purpose Stable Glove™) designed to support the
151 wrist and hand (and therefore could reduce strain injury in the operator) and a moveable metal
152 insert. The metal insert fingers were designed to fit around the bird's head to create a secure grip,
153 and to move independently from side to side in order to allow adjustment for different sizes of
154 birds (Figure 3). The rounded shape of the metal fingers was designed to aid the twisting motion

155 required to dislocate the bird's neck by enhancing the "rolling action" of the hand. The blunt
156 edge between the two metal fingers (protruding < 1 mm from the fleshy area of skin between the
157 index and middle fingers) provided a hard edge to force between the back of the bird's head and
158 the top of the neck, designed to focalise the force into the desired area (i.e. a dislocation at C0-
159 C1) when the method was applied.

160

161 *Figure 2*

162

163 *Figure 3*

164

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

170

171 Before this trial commenced, the modified devices had been tested in two previous experiments
172 and were applied to 80 cadavers (10 birds per bird type x age for each killing treatment), and 80
173 anaesthetised birds (10 birds per bird type x age for each killing treatment) that were subject to
174 detailed electroencephalography (EEG) analysis of electrical brain activity, reflex and
175 behavioural duration analysis and post mortem examination. These confirmed that both the
176 MZIN and MMCD caused tissue damage in the expected way that would be likely to result in

177 death, as well as causing rapid and sustained unconsciousness post device application (Martin
178 2015).

179

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

189

190 The efficacy of the devices was determined in two ways: (1) durations of reflexes post treatment
191 application; and (2) post mortem examination. Three cranial reflexes (pupillary (Croft 1961),
192 nictitating membrane (Erasmus *et al* 2010c; Heard 2000) and rhythmic breathing (Anil 1991;
193 Erasmus *et al* 2010a)) and four relevant involuntary behaviours (presence of jaw tone (Erasmus
194 *et al* 2010a; Sandercock *et al* 2014), cloacal movement (Erasmus *et al* 2010c), and clonic wing
195 flapping and leg paddling (Blackmore & Delany 1988; Erasmus *et al* 2010c; Gregory 1991))
196 (Table 2) were assessed as present or absent in 15 s intervals post killing treatments application,
197 until an uninterrupted 30 s of absence of all behaviours and reflexes was observed. Assessment
198 of the presence and absence of the behaviours and reflexes was conducted by two observers for

199 all birds: observer 1 assessed reflexes and behaviours associated with the bird's head, while
200 observer 2 assessed measures relating to the body and limbs of the bird. Measures were recorded
201 in a predetermined order for each observer, and using the 1-0 sampling technique (Martin &
202 Bateson 2007): if a reflex/behaviour was present during any point of a 15 s interval it was
203 defined as present for the entire interval, providing a conservative measure of reflex/behaviour
204 duration post killing treatment application. If a reflex or behaviour could not be recorded (e.g.
205 pupillary reflex – concealed due to damage to the eye) the data was recorded as missing.

206

207 *Table 2*

208

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

214

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

219

220 *Figure 4*

221

222 *Table 3*

223

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

230

231 Kill success was defined as only one application attempt with no signs of recovery (e.g.
232 sustained and/or return of rhythmic breathing and jaw tone, for example). If any signs of
233 recovery continued for 15 s (i.e. 1 interval measure) the bird was immediately emergency
234 euthanised; the method of euthanasia was killing treatment dependent in order to prevent post
235 mortem examination data being voided (e.g. for MCD and NMCD it was by the CASH Poultry
236 Killer .22 (CPK 200 – 1 grain (65 mg) gunpowder cartridge) (Accles & Shelvoke 2010); for
237 MZIN it was by MCD. Device success was defined as the killing treatments producing the
238 optimal trauma to the bird, specific to the treatment’s design. For example, the MZIN penetrating
239 the skull and causing more than one region of the brain a minimum of “mid” range damage, as
240 pilot work established this was sufficient to result in a successful kill. For the MCD and NMCD,

241 device success was defined as full dislocation of the neck at C0-C1, the spinal cord and both
242 carotid arteries severed and no tears or breaks to the skin (HSA 2004).

243

244 *Ethical statement*

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

249

250 *Statistical Analysis*

251 All data was summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat
252 (14th Edition). Statistical significance was termed by a threshold of 5% level and based on F
253 tests. A *P* value ranging from >0.05 - <0.10 was defined as a statistical trend. Summary graphs
254 and statistics were produced at the bird level. Statistical comparisons for kill success and device
255 success were conducted via Generalised Linear Mixed Models (GLMMs), using the logit link
256 function and binomial distribution.

257

258 Post-mortem measures were divided into neck damage methods (i.e. NMCD and MCD) and head
259 damage methods (MZIN) and analysed separately. Statistical comparisons were performed on
260 sub-sets of data to remove failure birds (i.e. kill success "no") in order to prevent data skewing.

261 All post-mortem binary measures (e.g. skin break yes/no) and categorised measures (e.g. brain

262 damage grade) were analysed via GLMMs using logit link function and binomial distribution.
263 Device success was used as a fixed effect within all the models.

264

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

274

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

278

279 **Results**

280 A total of 163 out of 180 birds were killed successfully by one of the three methods. Kill success
281 ($F_{2,167} = 19.96, P < 0.001$) and device success ($F_{2,167} = 7.33, P < 0.001$) were affected by killing
282 treatments, with NMCD and MCD achieving $100.0 \pm 0.0\%$ kill success rate and the MZIN

283 achieving $71.7 \pm 5.9\%$ (i.e. 17 birds were not killed successfully by the MZIN) . Device success
284 rates were NMCD = $41.7 \pm 6.4\%$; MZIN = $70.0 \pm 6.0\%$; and MCD = $26.7 \pm 5.8\%$. Kill order had
285 no effect on kill or device success. Bird type had an effect on device success ($F_{1,167} = 9.55, P =$
286 0.002), with device success being higher in broilers compared to layer birds, but there was also
287 an interaction between bird type and killing method ($F_{1,167} = 4.23, P = 0.036$) with device success
288 higher in the MZIN applied to broilers (Figure 5). Bird type had no effect on kill success,
289 although there was a significant interaction between killing treatments and bird type for kill
290 success ($F_{2,167} = 3.29, P = 0.040$) with the lowest kill success for layer type birds killed by MZIN
291 compared to broiler types, and with remaining killing treatments equally successful for killing
292 (100%), irrespective of bird type. Bird age, kill weight and all other interactions had no
293 significant effects on kill success or device success.

294

295 *Figure 5*

296

297 Of the birds killed successfully, means of the maximum duration times for cranial reflexes are
298 shown in Figure 6. Figures 6a and 6c demonstrate that there were no significant differences
299 between killing treatments in relation to mean of the maximum durations for nictitating
300 membrane and rhythmic breathing, but there was for pupillary reflex ($F_{1,150} = 101.66, P < 0.001$)
301 (Figure 6b), in which MZIN showed shorter maximum durations compared to NMCD and MCD
302 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
303 maximum pupillary durations, with layer (33.5 ± 2.5 s) and older (40.2 ± 5.7 s) birds showing
304 higher maximum pupillary durations compared to broilers (27.0 ± 2.2 s) and younger (22.5 ± 3.8

305 s) birds. Device success (yes or no) had an effect on pupillary maximum duration times (yes:
306 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
307 membrane maximum durations (yes: 2.3 ± 1.0 s; no: 3.6 ± 0.9 s) ($F_{1,150} = 3.86$, $P = 0.051$), with
308 both showing shorter maximum duration times for birds in which device success was achieved.
309 Nictitating membrane maximum durations were also affected by bird weight ($F_{1,150} = 5.09$, $P =$
310 0.025); and interactions between killing treatments and bird type ($F_{2,150} = 5.19$, $P = 0.007$); and
311 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)
312 and layer (3.6 ± 1.0 s) birds showing longer maximum durations compared to lighter (2.7 ± 1.0
313 s), younger (0.0 ± 0.0 s) and broiler (2.8 ± 1.0 s) birds.

314

315 *Figure 6*

316

317 For birds killed successfully, treatment affected the maximum durations of leg paddling, and
318 cloacal movement, but not wing flapping (which ranged 99-113 s). For leg paddling and cloacal
319 movement the NMCD device had the shortest mean of the maximum duration times (97.5 ± 5.6
320 s, 103.0 ± 6.1 s respectively) compared to the MCD (115.8 ± 6.8 s, 119.3 ± 6.9 s) and MZIN
321 (112.7 ± 7.1 s, 124.9 ± 6.3 s). Leg paddling, wing flapping and cloacal movement were all
322 affected by bird type and bird age (Table 4), with broilers and younger birds having shorter
323 maximum duration times compared to layers and older birds (Table 5). For cloacal movement
324 duration, bird weight also had an effect, with heavier birds exhibiting longer durations ($113.5 \pm$
325 7.5 s) compared to lighter birds (96.1 ± 9.8 s).

326

327 *Table 4*

328

329 *Table 5*

330

331 MZIN (0.3 ± 0.3 s) had significantly the shortest jaw tone duration compared to the NMCD and
332 MCD (8.8 ± 1.3 s; and 6.8 ± 1.3 s, respectively) (Table 4), but there was no significant difference
333 between the MCD and NMCD. Device success, bird type, bird age and bird weight did not
334 significantly affect jaw tone maximum durations. However, the interactions between kill
335 treatment and bird type; kill treatment and bird age; and bird age and kill weight were shown to
336 have an effect. The key differences relating to the interaction between kill treatment and bird
337 type were that the MZIN and NMCD showed that broilers had shorter jaw tone durations ($6.5 \pm$
338 1.7 s) compared to layers (11.0 ± 1.8 s), but the MCD showed no differences between bird types
339 (broiler = 6.5 ± 1.7 s; layer = 7.0 ± 1.9 s). The interaction between bird age and kill treatment
340 demonstrated that for the MCD and MZIN there were no differences between different bird ages
341 on jaw tone maximum durations. For the NMCD broiler chicks had the shortest jaw tone
342 durations (3.0 ± 1.6 s versus 8-14 s), but layer pullets were shown to have the longest durations
343 (14.0 ± 3.1 s), while broilers (slaughter age) and layer hens had no significant differences (range
344 8-10 s).

345

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

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

356

357 Table 6

358

359 Both the NMCD and the MCD caused subcutaneous hematomas in the neck, damage to the neck
360 muscle, cervical dislocation and spinal cord severance in 100% of successfully-killed birds ($n =$
361 60). A small proportion of birds showed minor tears to the skin (MCD – 6.7%; NMCD – 8.3%),
362 with fewer exhibiting external blood loss from the wounds (both 5%). There were no significant
363 effects of killing treatments on skin tears ($F_{1,103} = 0.12$, $P = 0.732$) or external blood loss ($F_{1,103} =$
364 0.00 , $P = 0.978$). There was no significant difference between the NMCD and MCD in terms of
365 dislocation position ($F_{1,103} = 0.79$, $P = 0.376$), with a C0-C1 dislocation level achieved in 85% of
366 birds for NMCD and 80% for MCD. The MCD attained the lowest break at C3-C4 in one bird.
367 Bird type ($F_{1,103} = 32.00$, $P < 0.001$) and bird age ($F_{1,103} = 32.14$, $P < 0.001$) had significant
368 effects on dislocation level, with layers and older birds more likely to be subject to lower

369 dislocations (\geq C1-C2) compared to broilers and younger birds. Dislocation level had no effect
370 on the maximum durations for all reflexes and behaviours.

371

372 The NMCD caused 0% vertebrae damage as a result of the dislocation, but the MCD caused
373 damage in 3.3% of birds, however the difference was not significant ($F_{1,103} = 2.02$, $P = 0.158$).

374 There was an interaction between killing treatments and bird age ($F_{2,103} = 4.43$, $P = 0.038$), with
375 two hens killed by the MCD method receiving damage to their vertebra.

376

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

385

386 The number of carotid arteries severed was affected by killing treatments ($F_{1,103} = 4.58$, $P =$
387 0.030), with the NMCD more likely to sever ≥ 1 carotid arteries compared to the MCD (means:
388 NMCD = 1.22 ± 0.11 ; MCD = 0.90 ± 0.11). The NMCD resulted in 71.7% of birds having ≥ 1
389 carotid arteries severed, compared to the MCD where only 58.3% of birds had ≥ 1 carotid arteries

390 severed. The number of carotid arteries severed was also affected by neck gap distance ($F_{1,103} =$
391 22.05, $P < 0.001$), with larger neck gap distances being positively associated with more carotid
392 arteries being severed. Bird type, age, weight and dislocation level did not affect the number of
393 carotid arteries severed (data not shown). The number of carotid arteries severed did not have a
394 significant effect on maximum durations of any of the reflexes and behaviours measured, apart
395 from having a tendency to affect jaw tone ($F_{2,102} = 2.53$, $P = 0.095$), in which severing zero or
396 one carotid artery did not affect maximum jaw tone durations (0 carotid arteries severed: MCD
397 7.2 ± 2.0 s and NMCD 9.7 ± 2.2 s; 1 carotid artery severed: MCD 8.4 ± 2.3 s and NMCD $12.6 \pm$
398 2.3 s), but if two were severed there was a reduction in maximum jaw tone duration (MCD $4.7 \pm$
399 2.3 s and NMCD 6.5 ± 2.3 s).

400

401 MZIN caused trauma to the head of the bird rather than the neck, therefore comparisons of post-
402 mortem trauma with NMCD and the MCD are not relevant. Kill success did not have significant
403 effect on broken skin, external bleeding and subcutaneous hematomas, with over 88% of birds
404 displaying these factors irrespective of kill success (Table 7). There was an effect of kill success
405 on skull damage ($F_{1,43} = 3.21$, $P = 0.024$), with more damage caused with successful kills, but
406 there was no effect in terms of where the skull was penetrated by the bolt ($F_{1,43} = 0.19$, $P =$
407 0.664). Device success had an affect on the location of bolt penetration into the skull, with birds
408 which achieved device success being more likely to have their skulls penetrated at locations CB
409 and CM (Figure 4); 79.1% of birds had damage in these two areas of the skull. The bird type,
410 age, weight and all interactions did not have an affect on the skull penetration area (data not
411 shown).

412

413 *Table 7*

414

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

422

423 More than 80% of birds killed successfully with the MZIN had damage (low mid or max) to all
424 main areas of the brain (Table 7 and Figure 7), excluding the brain stem, which was damaged in
425 just over 50% of birds. Kill success affected whether or not a brain region was damaged and the
426 grade of the damage. Damage to both sides of the forebrain, the cerebellum, and brain stem was
427 not affected by other factors (e.g. bird type, age, weight, interactions). Bird type had an effect on
428 damage to the midbrain, with layers more likely to sustain damage than broilers ($F_{1,43} = 6.03, P$
429 $= 0.014$). Only in successfully-killed birds did the highest grade of damage occur (max), with the
430 cerebellum sustaining the highest proportion of maximum damage. Following unsuccessful kills,
431 less than 45% of birds sustained brain damage and the brain stem was never damaged.

432

433 **Discussion**

434 This study evaluated the kill efficacy of three killing methods (MCD, NMCD, and MZIN) on
435 broilers and layers at two stages of production. Determining the kill efficacy of on-farm killing
436 methods involves three main considerations: reliability, humaneness and practicality. The
437 NMCD device and the MCD had kill success rates of 100%, compared to the 72% success rate of
438 the MZIN, and therefore were deemed the most reliable methods in this study. Other studies
439 have also demonstrated the high kill success rate in cervical dislocation methods (Erasmus *et al*
440 2010a; Erasmus *et al* 2010b; Gregory & Wotton 1990). Erasmus and colleagues (2010a) showed
441 that 100% of turkey hens (N = 26) were successfully killed by mechanical cervical dislocation,
442 re-enforcing the reliability of this method for killing poultry on-farm, but all of those birds
443 displayed a nictitating membrane reflex immediately post device application and maintained this
444 reflex for a mean of 106 s. However, the authors used a Burdizzo (a mechanical cervical
445 dislocation device), which is different to MCD and the NMCD, as it causes dislocation via
446 crushing, not through stretching and twisting (Erasmus *et al* 2010a). Crushing injury caused by
447 mechanical cervical dislocation methods is a cause for welfare concern as birds may die of
448 asphyxiation rather than cerebral ischemia, resulting in signs of consciousness for longer
449 (Gregory *et al* 1990). The use of the nictitating membrane as an indicator of insensibility has
450 been questioned, but it has been shown to be a more reliable indicator of complete brain death
451 (Anil 1991; Heard 2000; Sandercock *et al* 2014). Here, no more than 10% of birds ever showed
452 this reflex for any of the three killing treatments and the mean duration of those that did was > 5
453 s, suggesting that brain death occurred rapidly post-killing treatment application. Whether this is
454 rapid enough to be deemed humane is open to debate.

455

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

465

466 Despite the optimal kill success rate for the MCD and the NMCD, the device success rates were
467 significantly lower compared to that of the MZIN. With the MZIN, only 43/60 (72%) of birds
468 were successfully killed but 42 of those birds also achieved device success, therefore when the
469 method was applied correctly, it achieved an optimal effect on the bird. However, unsuccessful
470 killing of 28% of birds by the MZIN means that, despite its device success when it does kill, it is
471 an unacceptable method for killing poultry. Device success was greatly reduced for layer-type
472 birds compared to broilers for both the MCD and NMCD, which may be due to the more mature
473 skeleton and anatomy of the layer birds compared to the broilers, which would have made it
474 more difficult to dislocate the neck at higher points (e.g. C0-C1 or C1-C2), and therefore more
475 difficult to sever the spinal cord and carotid arteries, as with increasing age these vertebrae
476 become fused to the base of the skull and there is development of fibrous connective tissue
477 around it (McLeod *et al* 1964). MCD performed worst in terms of device success (27%) due to
478 the lower percentage of birds having both carotid arteries severed and fewer birds showing a

479 dislocation level of C0-C1 compared to the NMCD. Severing of one or more carotid arteries
480 causes a reduction in blood flow to the brain (Aslan *et al* 2006; Perry *et al* 2012; Whittow 2000)
481 and results in a reduction of arterial pressure and eventual cerebral ischemia and/or hypoxia
482 (Gregory & Wotton 1986; Gregory & Wotton 1990). However, even if the carotid arteries were
483 not completely severed, the stretching trauma results in narrowing and occlusion of the carotid
484 arteries which may have the same effect as severing them (LeBlang & Nunez 2000a; Whittow
485 2000). Both NMCD and MCD caused trauma to both carotid arteries, although did not always
486 sever them. This suggests that blood supply to the brain would be rapidly and significantly
487 reduced (LeBlang & Nunez 2000b; Perry *et al* 2012; Weir *et al* 2002), resulting in inability in the
488 brain to function correctly and the onset of neurogenic shock (Dumont *et al* 2001a), which could
489 be inferred as the bird not being fully conscious or suffering vasovagal episodes, as seen in
490 human cases of severe blood loss or restriction (Day *et al* 1982). Previous work has also
491 demonstrated that the higher up the carotid arteries are severed (e.g. at C0-C1 rather than C3-
492 C4), the less likely that false aneurysm formations and early arrested blood flow occurs (Gregory
493 *et al* 2012), both which could elongate the time to brain death. Several studies have also
494 highlighted the importance in severing both carotid arteries in exsanguination methods for
495 poultry as well as other livestock species in order to minimise the duration of brain activity
496 (Blackman *et al* 1986; Gregory *et al* 2012; Raj *et al* 2006). The same trauma should also reduce
497 the blood supply to the top of spinal cord, which causes functional impairment and could result
498 in neurogenic shock (Dumont *et al* 2001a; Dumont *et al* 2001b). The requirement to sever both
499 carotids may not be necessary to ensure that the ‘device’ or method can be considered successful,
500 providing sufficient stretching and twisting occurs, resulting in blood flow reduction to the brain.
501 The aim to achieve dislocation of the neck at C0-C1 was to ensure the damage and severing of

502 the spinal cord occurred very near to or at the brain stem, enhancing the likelihood of concussion
503 resulting in disruption to brain stem function and localised temporary or permanent biochemical
504 changes within the neural axons (Brieg 1970; Erasmus *et al* 2010b; Freeman & Wright 1953;
505 Krause *et al* 1988; Povlishock *et al* 1992; Takahashi *et al* 1981). More than 80% of birds killed
506 with both MCD and NMCD achieved a C0-C1 dislocation, so the likelihood of trauma to the
507 brain stem was high. Gregory & Wotton (1990) demonstrated that 6/8 birds culled by manual
508 cervical dislocation with dislocation at C0-C1 displayed a reduction in their visual evoked
509 responses, suggesting a loss of consciousness . The results of this study have demonstrated the
510 importance of attempting to sever both carotid arteries and dislocating as near to the skull as
511 possible (e.g. C0-C1), but that the stretch and twist damage was sufficient to kill the bird and
512 minimise the duration of consciousness-indicating reflexes post application (e.g. jaw tone,
513 nictitating membrane, and rhythmic breathing). Therefore the requirements for ‘device success’
514 may have been too strict in terms of resulting in a humane death, but could be be used as
515 guidance (i.e. gold standard) for optimal performance.

516

517 The damage caused by the MZIN to the bird’s head resulted in primary and secondary brain
518 injuries; causing brain contusions, haemorrhaging and axonal damage, all of which disrupt brain
519 function and can cause brain death (Claassen *et al* 2002; Kushner 1998; White & Krause 1993).
520 Successful kills by the MZIN resulted in extensive trauma to the forebrain and the cerebellum.
521 This affected the functioning of several systems e.g. motor systems (unconscious and conscious),
522 cognition, respiration and reflexes (Whittow 2000). The extent of axonal damage is correlated
523 with the amount of the brain damaged (Krause *et al* 1988; White & Krause 1993), therefore the
524 more extensive the brain damage, the more axons are damaged. Axonal damage has also been

525 linked to the length of concussion and unconsciousness (Kushner 1998; White & Krause 1993).
526 Skill was required to aim the device and successful judgment in applying reasonable force in
527 order to prevent the device re-coiling, as well as securing the bird's head in place. If this was not
528 achieved there was a reduction in the penetration depth of the bolt, which resulted in insufficient
529 brain damage to cause death. This is highlighted by the result that approximately 42% of birds
530 which were unsuccessfully killed by the device did not sustain any skull damage, as the head was
531 either missed completely or only a glancing blow was sustained, which caused only soft tissue
532 damage to the neck or eyes; or recoil resulted in insufficient power to penetrate the skull. The
533 MZIN required two operators, one to hold the bird, and other to cock and aim the device, as well
534 as a hard surface to rest the bird on, which could be deemed impractical in an on-farm situation.
535 There was also a health and safety concern with the device, as it is a captive bolt and therefore
536 great care is required during its use, and as such safety equipment must be worn (e.g. gloves,
537 safety goggles) (Pizzurro 2009a; Pizzurro 2009b). However, the primary issue with the MZIN
538 device was its low kill success rate of 72%, which is not reliable enough for a routine on-farm
539 killing method.

540

541 Durations of reflexes have been used and validated for inferring consciousness in killing
542 assessments of several animals, including poultry (Erasmus *et al* 2010a; Erasmus *et al* 2010b;
543 McKeegan *et al* 2013; Sandercock *et al* 2014). There were no significant differences between
544 killing methods on durations of rhythmic breathing and nictitating membrane and both were lost
545 within 3.4 s post-kill, suggesting both brain death and therefore unconsciousness occurred
546 rapidly for all killing methods. Loss of pupillary reflex is used as a conservative measure for
547 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.

569

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

591

592 The ceasing of clonic death-related behaviours (e.g. leg paddling and wing flapping) has been
593 used as an indicator of time of death for poultry which are killed by CO₂ gas stunning (Gerritzen
594 *et al* 2007), and based on this, all three killing methods were shown to kill birds in similar time
595 periods, despite small differences attributed to bird type and age, which may be indicative of
596 variation in bird nutrition and available muscle glycogen (Debut *et al* 2015; Petracci *et al* 2010).
597 The majority of birds showed convulsive wing flapping and leg paddling, which has been
598 observed in several other studies of killing with various methods (Abeyesinghe *et al* 2007;
599 Lambooij *et al* 1999; McKeegan *et al* 2007). The onset of cloacal movement, where visible, was
600 the last reflex observed before all movements ceased, which may highlight it as a conservative
601 indicator of death.

602

603 **Conclusion and Animal Welfare Implications**

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

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

616

617 **References**

618

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

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

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

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

629 **Aviagen** 2009 Ross 308 Broiler: Management Manual.

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

631 **Bader S, Meyer-Kühling B, Günther R, Breithaupt A, Rautenschlein S and Gruber A D**
632 2014 Anatomical and histologic pathology induced by cervical dislocation following blunt head
633 trauma for on-farm euthanasia of poultry. *Journal of Applied Poultry Research* 23: 546-556

634 **Bilello J F, Davis J W, Cunningham M A, Groom T F, Lemaster D and Sue L P** 2003
635 Cervical Spinal Cord Injury and the Need for Cardiovascular Intervention. *Arch Surg* 138: 1127-
636 1129

637 **Blackman N L, Cheetham K and Blackmore D K** 1986 Differences in blood supply to the
638 cerebral cortex between sheep and calves during slaughter. *Research in Veterinary Science* 40:
639 252-254

640 **Blackmore D K and Delany M W** 1988 Slaughter of Stock. A Practical Review and Guide. pp
641 1-134. Veterinary Continuing Education, Massey University: New Zealand

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

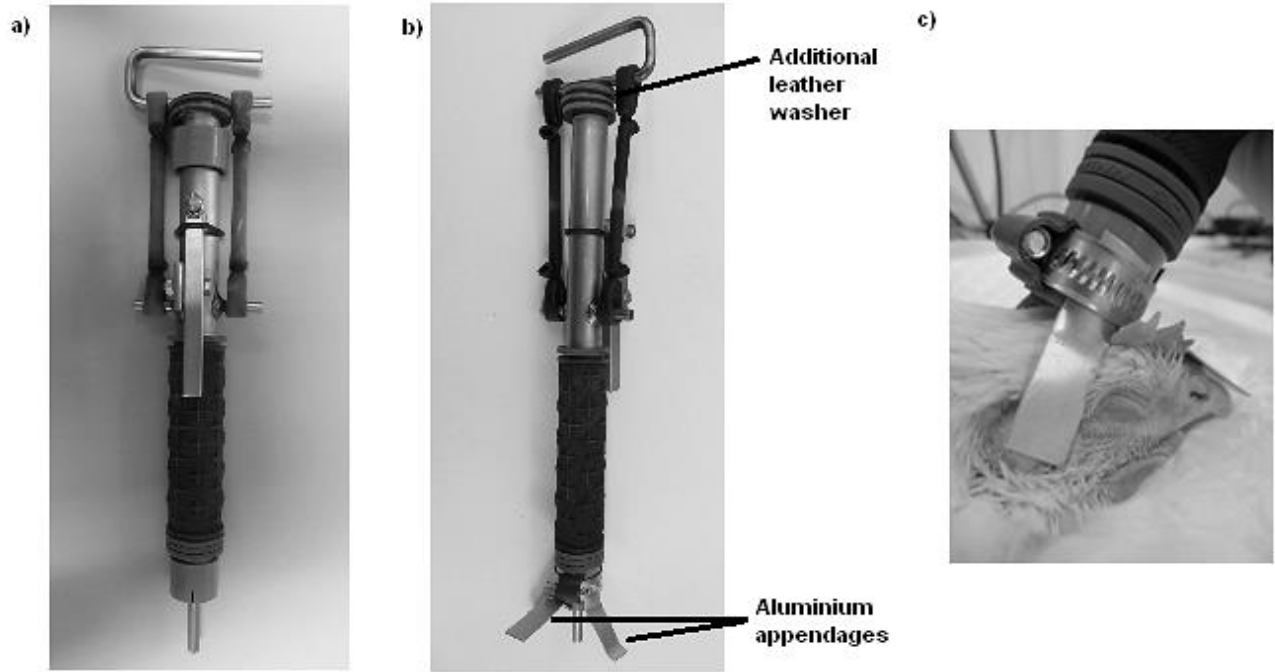
- 644 **Carbone L G, Carbone E T, Yi E M, Bauer D B, Lindstrom K A, Parker J M, Austin J A,**
645 **Seo Y, Gandhi A D and Wilkerson J D** 2012 Assessing cervical dislocation as a humane
646 euthanasia method for mice. *Journal of the American Association for Laboratory Animal Science*
647 *51*: 352-356
- 648 **Cartner S C, Barlow S C and Ness T J** 2007 Loss of Cortical Function in Mice After
649 Decapitation, Cervical Dislocation, Potassium Chloride Injection, and CO2 Inhalation.
650 *Comparative Medicine* *57*: 570-573
- 651 **Claassen J, Carhuapoma J R, Kreiter K T, Du E Y, Connolly E S and Mayer S A** 2002
652 Global cerebral edema after subarchnoid hemorrhage: frequency, predictors, and impact on
653 outcome. *Stroke* *33*: 1225-1232
- 654 **Close B, Banister K, Baumans V, Bernoth E, Bromage N, Bunyan J, Erhardt W, Flecknell**
655 **P, Gregory N G, Hackbarth H, Morton D and Warwick C** 2007 Recommendations for
656 euthanasia of experimental animals: Part 2. *Laboratory Animals* *31*: 1-32
- 657 **Cors J C, Gruber, A D, Günther R, Leyer-Kühling B, Esser K H, and Rautenschlein S** 2015
658 Electroencephalographic evaluation of the effectiveness of blunt trauma to induce loss of
659 consciousness for on-farm killing of chickens and turkeys. *Poultry Science* *00*: 1-9
- 660 **Croft P G** 1961 The photomotor reflex as an indicator of consciousness in the immobilized dog.
661 *Journal of Small Animal Practise* *2*: 206-214
- 662 **Dawson M D, Johnson K J, Benson E R, Alphin R L, Seta S and Malone G W** 2009
663 Determining cessation of brain activity during depopulation or euthanasia of broilers using
664 accelerometers. *Journal of Applied Poultry Research* *18*: 135-142
- 665 **Dawson M D, Lombardi M E, Benson E R, Alphin R L and Malone G W** 2007 Using
666 accelerometers to determine the cessation of activity in broilers. *Journal of Applied Poultry*
667 *Research* *16*: 583-591
- 668 **Day S, Cook E F, Funkenstein H and Goldman L** 1982 Evaluation and outcome of emergency
669 room patients with transient loss of consciousness. *The American Journal of Medicine* *73*: 15-23
- 670 **Debut M, Berri C, Arnould C, Guemené D, Santé-Lhoutellier V, Sellier N, Baéza E, Jehl N,**
671 **Jégo Y, Beaumont C, and Le Bihan-Duval E** 2015 Behavioural and physiological responses of
672 three chicken breeds to pre-slaughter shackling and acute head stress. *British Poultry Science*
673 *46(5)*:527-535
- 674 **DEFRA** 2002a Code of recommendations for the welfare of livestock: laying hens. Defra
675 Publications: London
- 676 **DEFRA** 2002b Codes of recommendations for the welfare of livestock:Meat chickens and
677 breeding chickens. Defra Publications: London

- 678 **DEFRA** 2014 DEFRA (MH0145) Welfare costs and benefits of existing and novel on-farm
679 culling methods for poultry. In:McKeegan, D E F, Martin, J E, Sandilands, V, Sandercock, D A,
680 Sparrey, J M and Sparks, N H C (eds) DEFRA Publications: UK
- 681 **Drew L B and Drew W E** 2004 The Contrecoup-Coup Phenomenon. *Neurocritical Care 1:*
682 385-390
- 683 **Dumont R J, Okonkwo D O, Verma S, Hurlbert R J, Boulos P T, Ellegala D B and Dumont**
684 **A S** 2001a Acute Spinal Cord Injury, Part I: Pathophysiologic Mechanisms. *Clinical*
685 *Neuropharmacology 24:* 254-264
- 686 **Dumont R J, Verma S, Okonkwo D O, Hurlbert R J, Boulos P T, Ellegala D B and Dumont**
687 **A S** 2001b Acute Spinal Cord Injury, Part II: Contemporary Pharmacotherapy. *Clinical*
688 *Neuropharmacology 24:* 265-279
- 689 **Erasmus M A, Lawlis P, Duncan I J H and Widowski T M** 2010a Using time insensibility
690 and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and
691 blunt trauma for on-farm killing of turkeys. *Poultry Science 89:* 1345-1354
- 692 **Erasmus M A, Turner P V, Nykamp S G and Widowski T M** 2010b Brain and skull lesions
693 resulting from use of percussive bolt, cervical dislocation by stretching, cervical dislocation by
694 crushing and blunt trauma in turkeys. *Veterinary Record 167:* 850-858
- 695 **Erasmus M A, Turner P V and Widowski T M** 2010c Measures of insensibility used to
696 determine effective stunning and killing of poultry. *Journal of Applied Poultry Research 19:*
697 288-298
- 698 **European Council** 2009 European Council Regulation (EC) 1099/2009 of 24 September 2009
699 on the protection of animals at the time of killing.
- 700 **Freeman L W and Wright T W** 1953 Experimental Observations of Concussion and
701 Contusions of the Spinal Cord. *Annals of Surgery 137:* 433-443
- 702 **Gerritzen M A, Lambooi H, Reimert H, Stegeman A and Spruijt B** 2007 A note on
703 behaviour of poultry exposed to increasing carbon dioxide concentrations. *Applied Animal*
704 *Behaviour Science 108:* 179-185
- 705 **Grandin T** 1994 Public veterinary medicine: food safety and handling. Euthanasia and slaughter
706 of livestock. *Journal of American Veterinary Medicine Association 212:* 39
- 707 **Gregory N G** 1991 Humane Slaughter. *Outlook on Agriculture 20:* 95-101
- 708 **Gregory N G, Schuster P, Mirabito L, Kolesar R and McManus T** 2012 Arrested blood flow
709 during false aneurysm formation in the carotid arteries of cattle slaughtered with and without
710 stunning. *Meat Science 90:* 368-372
- 711 **Gregory N G and Wotton S B** 1986 Effect of slaughter on the spontaneous and evoked activity
712 of the brain. *British Poultry Science 27:* 195-205

- 713 **Gregory N G and Wotton S B** 1990 Comparison of neck dislocation and percussion of the head
714 on visual evoked responses in the chicken's brain. *Veterinary Record* 126: 570-572
- 715 **Harrop J, Sharan A, Vaccaro A R and Przybylski G J** 2001 The Cause of Neurologic
716 Deterioration After Acute Cervical Spinal Cord Injury. *Spine* 26: 340-346
- 717 **Heard D** 2000 Perioperative supportive care and monitoring. *Vet.Clin.North*
718 *Am.Exot.Anim.Pract.* 3: 587-615
- 719 **Hellyer P W, Freeman L C and Hubbell J A E** 1991 Induction of Anesthesia with Diazepam-
720 Ketamine and Midazolam-Ketamine in Greyhounds. *Veterinary Surgery* 20: 143-147
- 721 **Hogg D A** 1982 Fusions occurring in the postcranial skeleton of the domestic fowl. *J Anat* 135:
722 501-512
- 723 **HSA** 2004 Practical Slaughter of Poultry: A Guide for the Small Producer. Humane Slaughter
724 Association:
- 725 **Hy-Line** 2012 Hy-Line Brown Performance Standards Manual. *2th edition*
- 726 **Krause G S, White B C and Aust S D** 1988 Brain cell death after ischemia and reperfusion: A
727 proposed biochemical sequence. *Crit Care Med* 16: 714-726
- 728 **Kushner D** 1998 Mild traumatic brain injury. *Arch Intern Med* 158: 1617-1624
- 729 **Lambooj E, Gerritzen M A, Engel B, Hillebrand S J W, Lankhaar J and Pieterse C** 1999
730 Behavioural responses during exposure of broiler chickens to different gas mixtures. *Applied*
731 *Animal Behaviour Science* 62: 255-265
- 732 **LeBlang S D and Nunez D B** 2000a Noninvasive imaging of cervical vascular injuries. *AJR*
733 *174*: 1269-1278
- 734 **LeBlang S D and Nunez D B** 2000b Noninvasive inaging of cervical vascular injuries.
735 *American Journal of Roentgenology* 174: 1269-1278
- 736 **Martin J E** 2015 Humane mechanical methods to kill poultry on-farm. *PhD Thesis* University of
737 Glasgow, UK
- 738 **Martin P and Bateson P** 2007 Measuring Behaviour. An Introductory Guide. *Cambridge*
739 *University Press* 92
- 740 **Mason C, Spence J, Bilbe L, Hughes T and Kirkwood J** 2009 Methods for dispatching
741 backyard poultry. *Veterinary Record* 164: 220
- 742 **McKeegan D E F, Abeyesinghe S M, McLeman M A, Lowe J C, Demmers T G M, Whiting**
743 **T L, Kranen R W, Van Bommel H, Lankhaar J A C and Wathes C M** 2007 Controlled
744 atmosphere stunning of broiler chickens. II. Effects on behvaiour, physiology and meat quality in
745 a commercial processing plant. *British Poultry Science* 48: 430-442

- 746 **McKeegan D E F, Reimert H G M, Hindle V A, Boulcott P, Sparrey J M and Gerritzen M**
747 **A** 2013 Physiological and behavioural responses of poultry exposed to gas filled high expansion
748 foam. *Poultry Science* 92: 1145-1154
- 749 **McLeod W M, Trotter D M and Lumb J W** 1964 Avian Anatomy. pp 19-20. Burgess
750 Publishing Company: United States of America
- 751 **Ommaya A K and Gennarelli T A** 1974 Cerebral concussion and traumatic unconsciousness.
752 *Brain* 97: 633-654
- 753 **Parent A, Harkey L H, Touchstone D A, Smit E E and Smith R R** 1992 Lateral Cervical
754 Spine Dislocation and Vertebral Artery Injury. *Neurosurgery* 31: 501-509
- 755 **Petracci M, Bianchi M, and Cavani C** 2010 Pre-slaughter handling and slaughtering factors
756 influencing poultry product quality. *World's Poultry Science Journal* 66(1): 17-26
- 757 **Perry M O, Snyder W H and Thal E R** 2012 Carotid artery injuries caused by blunt force
758 trauma. *Carotid Artery Injuries* 192: 74-77
- 759 **Pizzurro S** 2009a About us - expectation of order fulfilment.
- 760 **Pizzurro S** 2009b Zinger Stun GunsTM - The Rabbit ZingerTM, (TRZ001).
- 761 **Pryor J D and Shi R** 2006 Electrophysiological changes in isolated spinal cord white matter in
762 response to oxygen deprivation. *Spinal Cord* 44: 653-661
- 763 **Raj A B M, O'Callaghan M and Knowles T G** 2006 The effects of amount and frequency of
764 alternating current used in water bath stunning and of slaughter methods on
765 electroencephalograms in broilers. *Animal Welfare* 15: 7-18
- 766 **Sandercock D A, Auckburally A, Flaherty D, Sandilands V and McKeegan D E F** 2014
767 Avian reflex and electroencephalogram responses in different states of consciousness.
768 *Physiology & Behavior* 133: 252-259
- 769 **Sharma B R, Singh V P and Harish D** 2005 Neck Structure Injuries in Hanging - Comparing
770 Retrospective and Prospective Studies. *Med.Sci.Law* 45: 321-330
- 771 **Shaw N A** 2002 The neurophysiology of concussion. *Progress in Neurobiology* 67: 344
- 772 **Shi R and Pryor J D** 2002 Pathological Changes of Isolated Spinal Cord Axons in Respnse to
773 Mechanical Stretch. *Neuroscience* 110: 765-777
- 774 **Shi R and Whitebone J** 2006 Conduction Deficits and Membrane Disruption of Spinal Cord
775 Axons as a Function of Magnitude and Rate of Strain. *J Neurophysiol* 95: 3384-3390
- 776 **Silvano C, Gemma M, De Vitis A, Piccoli S, Frascoli C, and Beretta L** 1996 Difficult
777 Diagnosis of Laryngeal Blunt Trauma. *Journal of Trauma-Injury Infection & Critical Care* 40(5):
778 845-846

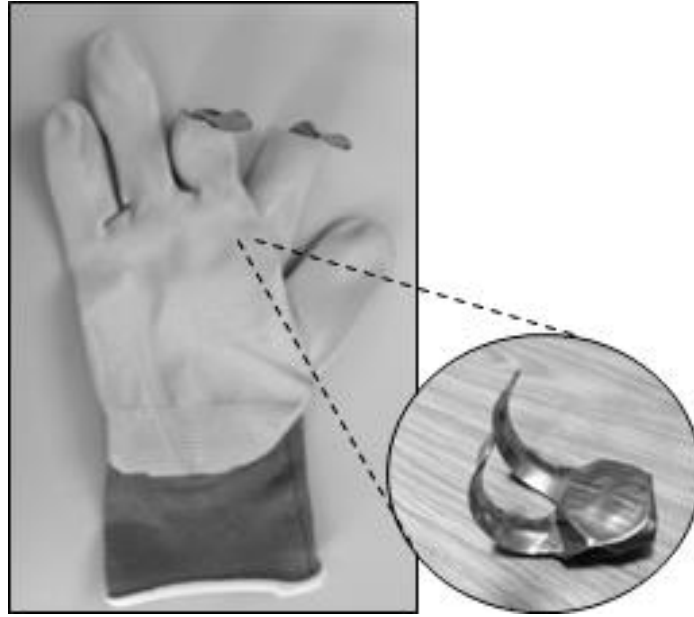
- 779 **Solomon** 1990 Human Anatomy and Physiology. pp 400-539. Saunders College Publishing:
- 780 **Sparrey J M, Sandercock D M, Sparks N H C and Sandilands V** 2014 Current and novel
781 methods for killing poultry individually on-farm. *World's Poultry Science Journal* 70(4) 737-758
- 782 **Takahashi H, Manaka S and Sano K** 1981 Changes of extracellular potassium concentration in
783 the cortex and brain stem during acute phase of experimental closed head injury. *No To Shinkei*
784 33: 365-376
- 785 **Tidswell S J, Blackmore D K and Newhook J C** 1987 Slaughter methods:
786 Electroencephalographs (EEG) studies on spinal cord section, decapitation and gross trauma of
787 the brain in lambs. *New Zealand Veterinary Journal* 35: 46-49
- 788 **Veras L, Pedraza-Gutiérrez S, Castellanos J, Capellades J, Casamitjana J and Rovira-**
789 **Cañellas A** 2000 Vertebral Artery Occlusion After Acute Cervical Spine Trauma. *Spine* 25:
790 1171-1177
- 791 **Weir C J, Zivin J A and Lyden P D** 2002 Inter-relationships between spinal cord blood flow,
792 neuronal death and neurological function in rabbit spinal cord ischemia. *Brain Research* 946: 43-
793 51
- 794 **White B C and Krause G S** 1993 Brain injury and repair mechanisms: the potential for
795 pharmacologic therapy in closed-head trauma. *Annals of Emergency Medicine* 22: 970-979
- 796 **Whittow G C** 2000 Sturkie's Avian Physiology. pp 71-80. Academic Press: UK
797
798



799

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

803



804

805

806

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

813

a)



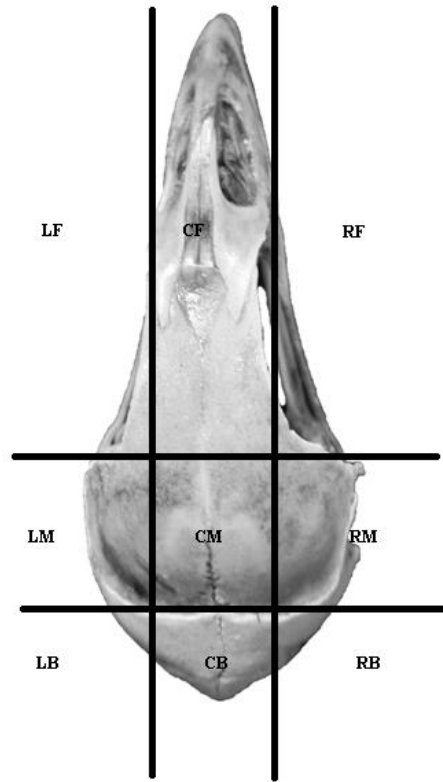
b)



814

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

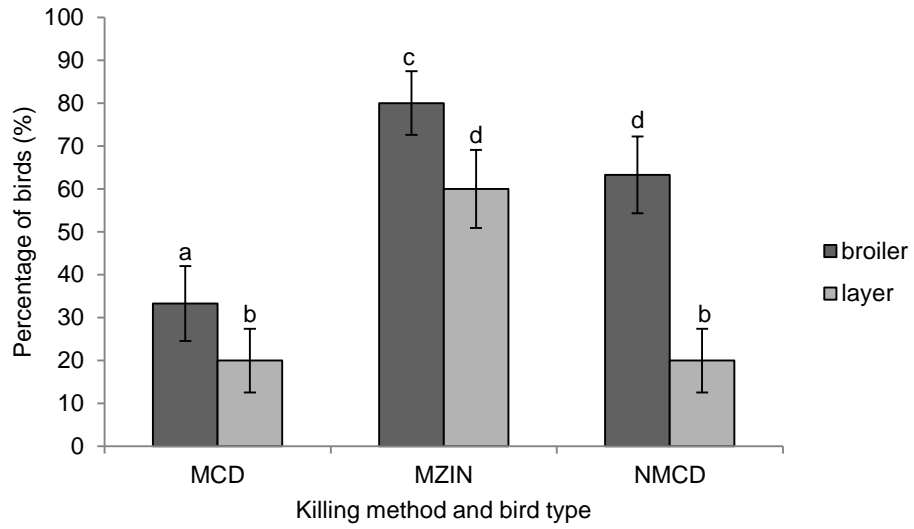
825



826

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

830

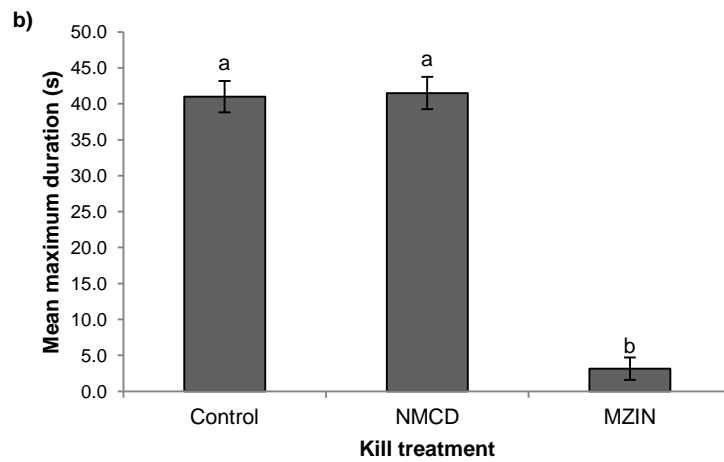
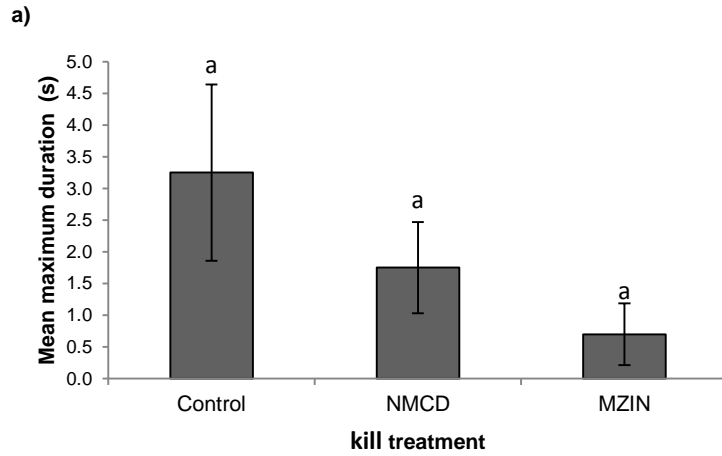


831

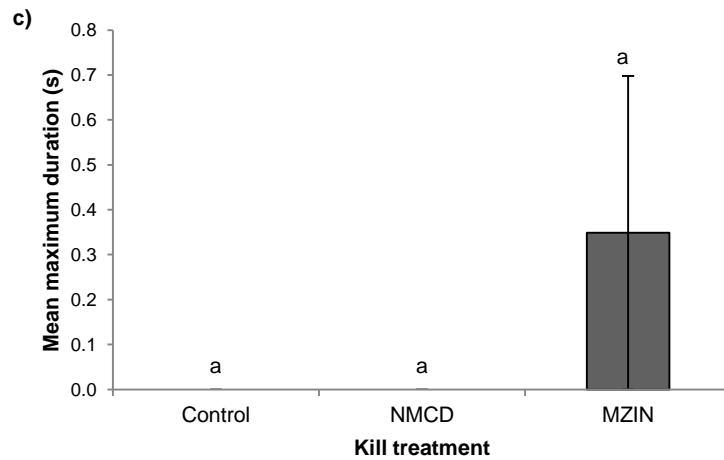
832 **Figure 5:** Mean (\pm SE) device success rates (%) across the three killing methods and bird type
 833 (broiler/layer).

834

835

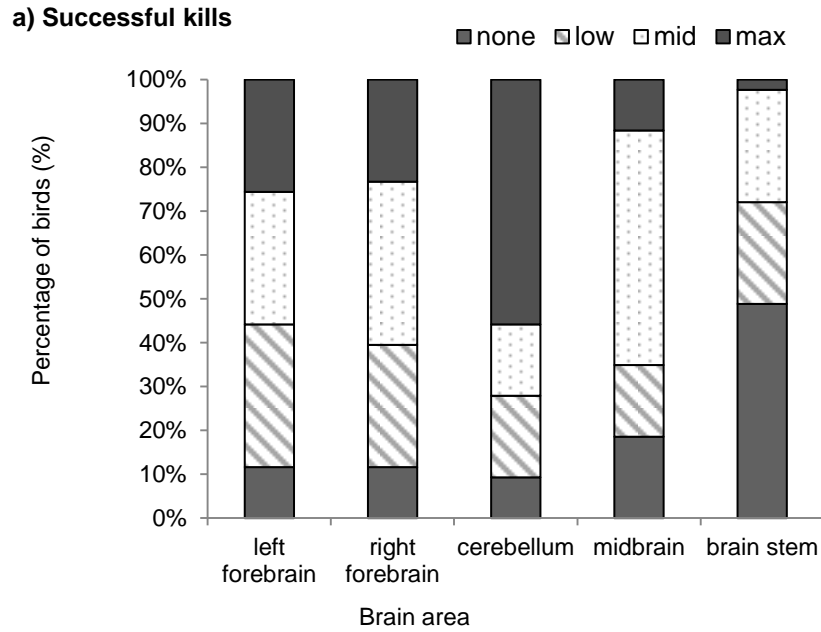


836

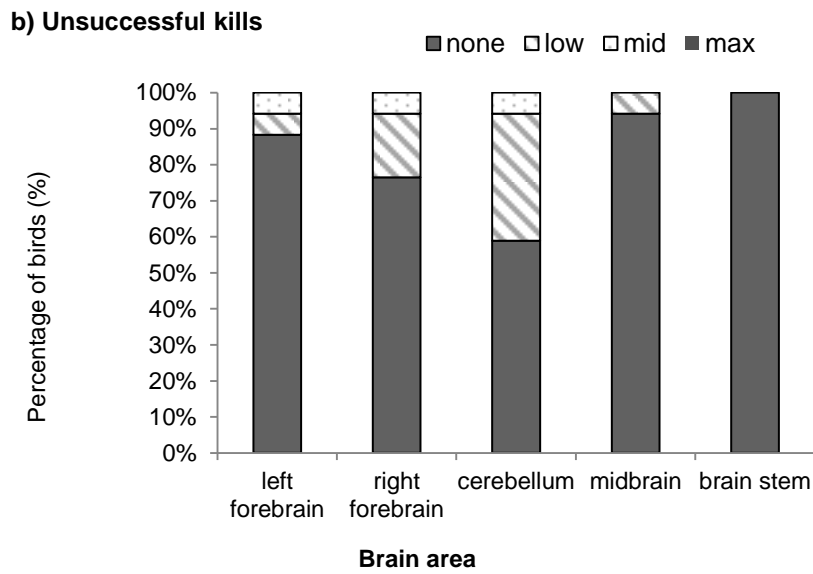


837

838 **Figure 6:** Mean (\pm SE) of the maximum durations (s) across the three killing treatments for the
839 cranial reflexes: a) nictitating membrane ($F_{2,150} = 1.67$, $P = 0.191$); b) pupillary ($F_{2,150} = 101.66$,
840 $P < 0.001$); and c) rhythmic breathing ($F_{2,150} = 1.46$, $P = 0.235$). Note that y axes ranges differ.



841



842

843 **Figure 7:** Comparison of brain damage ranges for successful and unsuccessful kills by the

844 MZIN. Refer to Table 3 for defined damage grading.

845

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.

846

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

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).

Fixed Effects	Wing flapping			Leg paddling		Cloacal movement		Jaw tone	
	<i>df</i>	<i>F</i> <i>statistic</i>	<i>P</i>	<i>F</i> <i>statistic</i>	<i>P</i>	<i>F</i> <i>statistic</i>	<i>P</i>	<i>F</i> <i>statistic</i>	<i>P</i>
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 $P < 0.05$.

Factor	Jaw tone		Wing flapping		Leg paddling		Cloacal movement	
	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

848

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

849

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

Post-mortem measure	Percentage of birds observed in (%)		<i>P</i> value
	Kill Success 'Yes'	Kill Success 'No'	
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

850

851
852