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

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14
15 **Abstract**

16 An alternative emergency method for killing poultry on-farm is required following European
17 legislation changes (EU 1099/2009), which heavily restricts the use of manual cervical dislocation.
18 This study investigated the kill efficacy of two mechanical methods that conform to the new
19 legislation: (1) a novel mechanical cervical dislocation device; and (2) a modified captive bolt
20 device (Rabbit Zinger™) and manual cervical dislocation (the control). Killing treatments were
21 applied to broilers and layers at two stages of production (broilers: 2-3 weeks and 5 weeks of age;

22 layers: 12-13 weeks and 58-62 weeks), with a total of 180 birds. Latency to abolition of cranial
23 and behavioural reflexes, as well as post-mortem analysis of the physiological damage produced,
24 were used to estimate time to unconsciousness and assess kill efficacy. The novel mechanical
25 cervical dislocation device was reliable and a practical method for killing poultry on-farm, with
26 100% kill success ($F_{2,167} = 19.96, P < 0.001$) and cranial reflex interval mean durations lasting for
27 0.0 - 103.0 s post-kill (jaw tone ($F_{2,150} = 13.34, P < 0.001$); pupillary ($F_{2,150} = 101.66, P < 0.001$);
28 nictitating membrane ($F_{2,150} = 1.61, P = 0.191$); and rhythmic breathing ($F_{2,150} = 1.46, P = 0.235$)
29 compared to the modified Rabbit ZingerTM (72% kill success rate, 0.3-124.9 s cranial reflex mean
30 durations) and manual cervical dislocation (100% kill success rate, 0.0-119.3 s cranial reflex mean
31 durations). The novel mechanical cervical dislocation device resulted in consistent anatomical
32 damage to the birds (e.g. high dislocation of the neck and severing of the spinal cord) compared to
33 the manual method, despite both having 100% success rate, while the modified Rabbit ZingerTM
34 was difficult to operate and resulted in varied anatomical damage. The novel mechanical cervical
35 dislocation device showed promise as 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 slaughter
46 and then slaughtered using gas or electrical waterbath stunning methods. However, for individual
47 birds on-farm, there are two key methods for killing poultry: (1) cervical dislocation, which is
48 designed to cause death by cerebral ischemia and extensive damage to the spinal cord and brain
49 stem (Bader *et al* 2014; Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory & Wotton 1990;
50 Ommaya & Gennarelli 1974); and (2) percussive devices designed to cause extensive brain
51 damage, resulting in brain death (Erasmus *et al* 2010a; Erasmus *et al* 2010b; Gregory & Wotton
52 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 dislocation
55 of the neck by hand (MCD); and (2) mechanical – cervical dislocation of the neck with the aid of
56 a tool (Gregory & Wotton 1990; HSA 2004; Mason *et al* 2009; Sparrey *et al* 2014). The most
57 common method for despatching poultry on-farm is manual cervical dislocation (MCD) (Mason
58 *et al* 2009), as it is perceived to be humane by users, easy to learn and perform, and does not
59 require equipment. All cervical dislocation killing methods are designed to separate the skull from
60 the vertebral column of the bird (C0-C1 vertebral dislocation), resulting in severing of the spinal
61 cord and/or brain stem and the main blood vessels supplying the brain (Cartner *et al* 2007; Gregory
62 & Wotton 1990; Mason *et al* 2009; Parent *et al* 1992; Veras *et al* 2000). It has been suggested that
63 optimal application also produces a concussive effect on the bird due to trauma inflicted on the
64 brain stem through the action of stretching and twisting (Cartner *et al* 2007; Erasmus *et al* 2010a;
65 Harrop *et al* 2001; Pryor & Shi 2006; Shi & Pryor 2002; Shi & Whitebone 2006). However, both

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

79

80 Assessing the effectiveness and humaneness of a kill method is achieved, in part, by determining
81 time to unconsciousness (insensibility) and brain death. Several studies have identified and
82 validated the loss of brain stem (e.g. corneal) and spinal (e.g. nociceptive) reflexes as an indicator
83 of loss of consciousness, and/or brain death in poultry (Erasmus *et al* 2010a; McKeegan *et al* 2013;
84 Sandercock *et al* 2014; Sparrey *et al* 2014), as well as in several other species (Croft 1961; Hellyer
85 *et al* 1991). The loss of pupillary reflex and jaw tone have both been used as indicators of
86 unconsciousness. Some studies have also used the cessation of clonic convulsions in poultry (e.g.
87 wing-flapping and leg paddling) as an indication of brain death (Dawson *et al* 2009; Dawson *et al*
88 2007), as well as cessation of rhythmic breathing (Blackmore & Delany 1988; Erasmus *et al* 2010a;

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

93

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

97

98 **Materials and Methods**

99 *Animal housing and husbandry*

100 A total of 180 female chickens were used for the study. The birds were tested in two batches of 90
101 birds on separate days. In each batch 15 layers and 15 broilers, each divided into two age classes
102 (either 7 or 8 birds per type/age-class depending on the day tested, but always totalling 15 over the
103 two days), were assessed for each of the three killing treatments (N=15). Further details about the
104 birds and their accommodation are provided in Table 1. The sample size was chosen to allow
105 significant differences to be identified in behavioural data which is prone to high individual
106 variation across two bird types and two bird age groups (within type) across three killing
107 treatments. A minimum of 12 birds were calculated to provide sufficient power in the analysis
108 (88%), however an additional 3 birds were used per treatment group in order to compensate for
109 any unsuccessful birds and therefore the loss of valid behavioural data.

110

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

121

122 *Table 1*

123

124 ***Study design***

125 Two novel mechanical poultry killing devices, the Modified Rabbit Zinger (MZIN) and a novel
126 mechanical cervical dislocation gloved device (NMCD), were assessed for their kill efficacy in
127 comparison with each other and a control (MCD). The Rabbit ZingerTM (Pizzurro 2009a; Pizzurro
128 2009b) is a penetrating captive bolt device originally designed to kill rabbits that uses the stored
129 energy in rubber tubes to drive a penetrating bolt into the head, causing death by extensive
130 irreversible brain damage (DEFRA 2014; Martin 2015) (Figure 1a). The device was modified with
131 permission of the original designer in order to adapt it to the new target species (Figure 1b),

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

143

144 *Figure 1*

145

146 The NMCD device (Figure 2) was designed to create a mechanical method for cervical dislocation
147 of poultry which mirrored the technique of the manual method. The device consisted of a
148 supportive glove (SHOWA 370 Multi-purpose Stable Glove™) designed to support the wrist and
149 hand (and therefore could reduce strain injury in the operator) and a moveable metal insert. The
150 metal insert fingers were designed to fit around the bird's head to create a secure grip, and to move
151 independently from side to side in order to allow adjustment for different sizes of birds (Figure 3).
152 The rounded shape of the metal fingers was designed to aid the twisting motion required to
153 dislocate the bird's neck by enhancing the "rolling action" of the hand. The blunt edge between
154 the two metal fingers (protruding < 1 mm from the fleshy area of skin between the index and

155 middle fingers) provided a hard edge to force between the back of the bird's head and the top of
156 the neck, designed to focalise the force into the desired area (i.e. a dislocation at C0-C1) when the
157 method was applied.

158

159 *Figure 2*

160

161 *Figure 3*

162

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

168

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

176

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

186

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

198 predetermined order for each observer, and using the 1-0 sampling technique (Martin & Bateson
199 2007): if a reflex/behaviour was present during any point of a 15 s interval it was defined as present
200 for the entire interval, providing a conservative measure of reflex/behaviour duration post killing
201 treatment application. If a reflex or behaviour could not be recorded (e.g. pupillary reflex –
202 concealed due to damage to the eye) the data was recorded as missing.

203

204 *Table 2*

205

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

211

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

216

217 *Figure 4*

218

219 *Table 3*

220

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

227

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

240

241 ***Ethical statement***

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

246

247 ***Statistical Analysis***

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

254

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

261

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

271

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

275

276 **Results**

277 A total of 163 out of 180 birds were killed successfully by one of the three methods. Kill success
278 ($F_{2,167} = 19.96, P < 0.001$) and device success ($F_{2,167} = 7.33, P < 0.001$) were affected by killing
279 treatments, with NMCD and MCD achieving $100.0 \pm 0.0\%$ kill success rate and the MZIN
280 achieving $71.7 \pm 5.9\%$ (i.e. 17 birds were not killed successfully by the MZIN) . Device success
281 rates were NMCD = $41.7 \pm 6.4\%$; MZIN = $70.0 \pm 6.0\%$; and MCD = $26.7 \pm 5.8\%$. Kill order had

282 no effect on kill or device success. Bird type had an effect on device success ($F_{1,167} = 9.55, P =$
283 0.002), with device success being higher in broilers compared to layer birds, but there was also an
284 interaction between bird type and killing method ($F_{1,167} = 4.23, P = 0.036$) with device success
285 higher in the MZIN applied to broilers (Figure 5). Bird type had no effect on kill success, although
286 there was a significant interaction between killing treatments and bird type for kill success ($F_{2,167}$
287 $= 3.29, P = 0.040$) with the lowest kill success for layer type birds killed by MZIN compared to
288 broiler types, and with remaining killing treatments equally successful for killing (100%),
289 irrespective of bird type. Bird age, kill weight and all other interactions had no significant effects
290 on kill success or device success.

291

292 *Figure 5*

293

294 Of the birds killed successfully, means of the maximum duration times for cranial reflexes are
295 shown in Figure 6. Figures 6a and 6c demonstrate that there were no significant differences
296 between killing treatments in relation to mean of the maximum durations for nictitating membrane
297 and rhythmic breathing, but there was for pupillary reflex ($F_{1,150} = 101.66, P < 0.001$) (Figure 6b),
298 in which MZIN showed shorter maximum durations compared to NMCD and MCD birds. Bird
299 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
300 pupillary durations, with layer (33.5 ± 2.5 s) and older (40.2 ± 5.7 s) birds showing higher
301 maximum pupillary durations compared to broilers (27.0 ± 2.2 s) and younger (22.5 ± 3.8 s) birds.
302 Device success (yes or no) had an effect on pupillary maximum duration times (yes: 20.1 ± 2.5 s;
303 no: 39.0 ± 1.8 s) ($F_{1,150} = 6.10, P = 0.015$) and a tendency to affect nictitating membrane maximum

304 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
305 maximum duration times for birds in which device success was achieved. Nictitating membrane
306 maximum durations were also affected by bird weight ($F_{1,150} = 5.09$, $P = 0.025$); and interactions
307 between killing treatments and bird type ($F_{2,150} = 5.19$, $P = 0.007$); and bird age and bird weight
308 ($F_{2,150} = 7.04$, $P < 0.001$), with heavier (3.3 ± 1.0 s), older (1.96 ± 1.1 s) and layer (3.6 ± 1.0 s)
309 birds showing longer maximum durations compared to lighter (2.7 ± 1.0 s), younger (0.0 ± 0.0 s)
310 and broiler (2.8 ± 1.0 s) birds.

311

312 *Figure 6*

313

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

323

324 *Table 4*

325

326 *Table 5*

327

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

341

342 The percentage of successfully-killed birds that exhibited various reflexes and involuntary
343 behaviours varied by killing treatments, although the MCD and NMCD were similar (Table 6).
344 For nictitating membrane and pupillary reflexes, both the MCD and NMCD had numerically
345 higher percentages of birds displaying these reflexes post-kill compared to MZIN, but these were
346 not significant. However, the MZIN was the only killing treatment in which a single bird showed

347 rhythmic breathing following a successful kill. In all killing treatments the majority of birds
348 displayed convulsive behaviours post-application (e.g. wing flapping and leg paddling) and the
349 last behaviour to cease was cloacal movement. Cloacal movement was not observed in a small
350 number of birds (7 birds of successful kills), however this was due to the birds defecating and the
351 movement being hidden as a result.

352

353 Table 6

354

355 Both the NMCD and the MCD caused subcutaneous hematomas in the neck, damage to the neck
356 muscle, cervical dislocation and spinal cord severance in 100% of successfully-killed birds (n =
357 60). A small proportion of birds showed minor tears to the skin (MCD – 6.7%; NMCD – 8.3%),
358 with fewer exhibiting external blood loss from the wounds (both 5%). There were no significant
359 effects of killing treatments on skin tears ($F_{1,103} = 0.12, P = 0.732$) or external blood loss ($F_{1,103} =$
360 $0.00, P = 0.978$). There was no significant difference between the NMCD and MCD in terms of
361 dislocation position ($F_{1,103} = 0.79, P = 0.376$), with a C0-C1 dislocation level achieved in 85% of
362 birds for NMCD and 80% for MCD. The MCD attained the lowest break at C3-C4 in one bird.
363 Bird type ($F_{1,103} = 32.00, P < 0.001$) and bird age ($F_{1,103} = 32.14, P < 0.001$) had significant effects
364 on dislocation level, with layers and older birds more likely to be subject to lower dislocations (\geq
365 C1-C2) compared to broilers and younger birds. Dislocation level had no effect on the maximum
366 durations for all reflexes and behaviours.

367

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

372

373 Gap distance between the two points of dislocation was significantly affected by killing treatments
374 ($F_{1,103} = 7.65$, $P = 0.007$) and bird weight ($F_{1,103} = 25.39$, $P < 0.001$). The NMCD method was
375 more likely to result in a larger gap distance compared to the MCD (6.29 ± 0.27 cm and $5.47 \pm$
376 0.21 cm respectively). Heavier birds were more likely to have large neck gap distances compared
377 to lighter birds (6.8 ± 0.38 cm and 4.9 ± 0.41 cm respectively). Bird type, bird age, dislocation
378 level and all interactions did not affect gap distances (data not shown). The maximum neck gap
379 sizes for each killing treatments were 9.0 cm for MCD and 10.0 cm for NMCD.

380

381 The number of carotid arteries severed was affected by killing treatments ($F_{1,103} = 4.58$, $P = 0.030$),
382 with the NMCD more likely to sever ≥ 1 carotid arteries compared to the MCD (means: NMCD =
383 1.22 ± 0.11 ; MCD = 0.90 ± 0.11). The NMCD resulted in 71.7% of birds having ≥ 1 carotid arteries
384 severed, compared to the MCD where only 58.3% of birds had ≥ 1 carotid arteries severed. The
385 number of carotid arteries severed was also affected by neck gap distance ($F_{1,103} = 22.05$, $P <$
386 0.001), with larger neck gap distances being positively associated with more carotid arteries being
387 severed. Bird type, age, weight and dislocation level did not affect the number of carotid arteries
388 severed (data not shown). The number of carotid arteries severed did not have a significant effect
389 on maximum durations of any of the reflexes and behaviours measured, apart from having a

390 tendency to affect jaw tone ($F_{2,102} = 2.53, P = 0.095$), in which severing zero or one carotid artery
391 did not affect maximum jaw tone durations (0 carotid arteries severed: MCD 7.2 ± 2.0 s and
392 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
393 were severed there was a reduction in maximum jaw tone duration (MCD 4.7 ± 2.3 s and NMCD
394 6.5 ± 2.3 s).

395

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

406

407 *Table 7*

408

409 Irrespective of kill success, 64% of birds sustained an internal brain cavity hematoma after
410 application of MZIN (Table 7). Kill success had an affect on the presence of an internal brain

411 cavity hematoma ($F_{1,43} = 5.57, P = 0.018$), with successfully killed birds more likely to have
412 bleeding within the skull. Device success, bird type and all interactions did not have significant
413 effects. Bird age ($F_{1,43} = 16.47, P < 0.001$) and weight ($F_{1,43} = 19.09, P < 0.001$) had effects on
414 tissue damage, with heavier and older birds more likely to have internal brain cavity hematomas,
415 compared to lighter and younger birds.

416

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

426

427 **Discussion**

428 This study evaluated the kill efficacy of three killing methods (MCD, NMCD, and MZIN) on
429 broilers and layers at two stages of production. Determining the kill efficacy of on-farm killing
430 methods involves three main considerations: reliability, humaneness and practicality. The NMCD
431 device and the MCD had kill success rates of 100%, compared to the 72% success rate of the
432 MZIN, and therefore were deemed the most reliable methods in this study. Other studies have also

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

449

450 When the NMCD and MCD were applied, they did not require precision aiming, unlike the MZIN,
451 which meant that a kill success was easier to achieve. MCD does not require any equipment and
452 once trained is relatively simple to apply on birds under 3 kg (HSA 2004). The NMCD glove
453 provided the correct position to hold the bird's head in place to perform the stretch and twisting
454 action, which for an inexperienced individual may be beneficial. Therefore the presence of the
455 glove did not hinder the application of the technique, as both MCD and NMCD had 100% kill

456 success rate. All birds that underwent MCD or NMCD immediately wing flapped and leg paddled
457 vigorously post-application and an obvious internal gap in the neck, between two cervical
458 vertebrae could be felt.

459

460 Despite the optimal kill success rate for the MCD and the NMCD, the device success rates were
461 significantly lower compared to that of the MZIN. With the MZIN, only 43/60 (72%) of birds were
462 successfully killed but 42 of those birds also achieved device success, therefore when the method
463 was applied correctly, it achieved an optimal effect on the bird. However, unsuccessful killing of
464 28% of birds by the MZIN means that, despite its device success when it does kill, it is an
465 unacceptable method for killing poultry. Device success was greatly reduced for layer-type birds
466 compared to broilers for both the MCD and NMCD, which may be due to the more mature skeleton
467 and anatomy of the layer birds compared to the broilers, which would have made it more difficult
468 to dislocate the neck at higher points (e.g. C0-C1 or C1-C2), and therefore more difficult to sever
469 the spinal cord and carotid arteries, as with increasing age these vertebrae become fused to the
470 base of the skull and there is development of fibrous connective tissue around it (McLeod *et al*
471 1964). MCD performed worst in terms of device success (27%) due to the lower percentage of
472 birds having both carotid arteries severed and fewer birds showing a dislocation level of C0-C1
473 compared to the NMCD. Severing of one or more carotid arteries causes a reduction in blood flow
474 to the brain (Aslan *et al* 2006; Perry *et al* 2012; Whittow 2000) and results in a reduction of arterial
475 pressure and eventual cerebral ischemia and/or hypoxia (Gregory & Wotton 1986; Gregory &
476 Wotton 1990). However, even if the carotid arteries were not completely severed, the stretching
477 trauma results in narrowing and occlusion of the carotid arteries which may have the same effect
478 as severing them (LeBlang & Nunez 2000a; Whittow 2000). Both NMCD and MCD caused trauma

479 to both carotid arteries, although did not always sever them. This suggests that blood supply to the
480 brain would be rapidly and significantly reduced (LeBlang & Nunez 2000b; Perry *et al* 2012; Weir
481 *et al* 2002), resulting in inability in the brain to function correctly and the onset of neurogenic
482 shock (Dumont *et al* 2001a), which could be inferred as the bird not being fully conscious or
483 suffering vasovagal episodes, as seen in human cases of severe blood loss or restriction (Day *et al*
484 1982). Previous work has also demonstrated that the higher up the carotid arteries are severed (e.g.
485 at C0-C1 rather than C3-C4), the less likely that false aneurysm formations and early arrested
486 blood flow occurs (Gregory *et al* 2012), both which could elongate the time to brain death. Several
487 studies have also highlighted the importance in severing both carotid arteries in exsanguination
488 methods for poultry as well as other livestock species in order to minimise the duration of brain
489 activity (Blackman *et al* 1986; Gregory *et al* 2012; Raj *et al* 2006). The same trauma should also
490 reduce the blood supply to the top of spinal cord, which causes functional impairment and could
491 result in neurogenic shock (Dumont *et al* 2001a; Dumont *et al* 2001b). The requirement to sever
492 both carotids may not be necessary to ensure that the ‘device’ or method can be considered
493 successful, providing sufficient stretching and twisting occurs, resulting in blood flow reduction
494 to the brain. The aim to achieve dislocation of the neck at C0-C1 was to ensure the damage and
495 severing of the spinal cord occurred very near to or at the brain stem, enhancing the likelihood of
496 concussion resulting in disruption to brain stem function and localised temporary or permanent
497 biochemical changes within the neural axons (Brieg 1970; Erasmus *et al* 2010b; Freeman &
498 Wright 1953; Krause *et al* 1988; Povlishock *et al* 1992; Takahashi *et al* 1981). More than 80% of
499 birds killed with both MCD and NMCD achieved a C0-C1 dislocation, so the likelihood of trauma
500 to the brain stem was high. Gregory & Wotton (1990) demonstrated that 6/8 birds culled by manual
501 cervical dislocation with dislocation at C0-C1 displayed a reduction in their visual evoked

502 responses, suggesting a loss of consciousness . The results of this study have demonstrated the
503 importance of attempting to sever both carotid arteries and dislocating as near to the skull as
504 possible (e.g. C0-C1), but that the stretch and twist damage was sufficient to kill the bird and
505 minimise the duration of consciousness-indicating reflexes post application (e.g. jaw tone,
506 nictitating membrane, and rhythmic breathing). Therefore the requirements for ‘device success’
507 may have been too strict in terms of resulting in a humane death, but could be used as guidance
508 (i.e. gold standard) for optimal performance.

509
510 The damage caused by the MZIN to the bird’s head resulted in primary and secondary brain
511 injuries; causing brain contusions, haemorrhaging and axonal damage, all of which disrupt brain
512 function and can cause brain death (Claassen *et al* 2002; Kushner 1998; White & Krause 1993).
513 Successful kills by the MZIN resulted in extensive trauma to the forebrain and the cerebellum.
514 This affected the functioning of several systems e.g. motor systems (unconscious and conscious),
515 cognition, respiration and reflexes (Whittow 2000). The extent of axonal damage is correlated with
516 the amount of the brain damaged (Krause *et al* 1988; White & Krause 1993), therefore the more
517 extensive the brain damage, the more axons are damaged. Axonal damage has also been linked to
518 the length of concussion and unconsciousness (Kushner 1998; White & Krause 1993). Skill was
519 required to aim the device and successful judgment in applying reasonable force in order to prevent
520 the device re-coiling, as well as securing the bird’s head in place. If this was not achieved there
521 was a reduction in the penetration depth of the bolt, which resulted in insufficient brain damage to
522 cause death. This is highlighted by the result that approximately 42% of birds which were
523 unsuccessfully killed by the device did not sustain any skull damage, as the head was either missed
524 completely or only a glancing blow was sustained, which caused only soft tissue damage to the

525 neck or eyes; or recoil resulted in insufficient power to penetrate the skull. The MZIN required
526 two operators, one to hold the bird, and other to cock and aim the device, as well as a hard surface
527 to rest the bird on, which could be deemed impractical in an on-farm situation. There was also a
528 health and safety concern with the device, as it is a captive bolt and therefore great care is required
529 during its use, and as such safety equipment must be worn (e.g. gloves, safety goggles) (Pizzurro
530 2009a; Pizzurro 2009b). However, the primary issue with the MZIN device was its low kill success
531 rate of 72%, which is not reliable enough for a routine on-farm killing method.

532

533 Durations of reflexes have been used and validated for inferring consciousness in killing
534 assessments of several animals, including poultry (Erasmus *et al* 2010a; Erasmus *et al* 2010b;
535 McKeegan *et al* 2013; Sandercock *et al* 2014). There were no significant differences between
536 killing methods on durations of rhythmic breathing and nictitating membrane and both were lost
537 within 3.4 s post-kill, suggesting both brain death and therefore unconsciousness occurred rapidly
538 for all killing methods. Loss of pupillary reflex is used as a conservative measure for brain death
539 and complete insensibility (Erasmus *et al* 2010c; Heard 2000; Sandercock *et al* 2014), and the
540 MZIN had the shortest durations for pupillary reflex compared to NMCD and the MCD, however
541 this only occurred in birds killed successfully with the MZIN which was low. Such low reliability
542 of successful kills means that the MZIN cannot be considered to be humane. The shorter duration
543 of the pupillary reflex for the MZIN may be explained by the type and location of trauma the kill
544 treatment caused. The bolt of the MZIN damaged the midbrain in more than 80% of birds; the
545 midbrain is reported to be the area within the brain that controls the nictitating membrane, as well
546 as the pupillary reflex (Solomon 1990; Whittow 2000), therefore direct trauma to it would result
547 in impairment of these reflexes. Damage to the surrounding areas of the brain could also cause

548 indirect trauma to the midbrain (e.g. contrecoup damage) and therefore impair reflexes (Drew &
549 Drew 2004; White & Krause 1993). Mature layer hens (irrespective of age) exhibited longer
550 durations for pupillary reflex when killed with MZIN compared to broilers, which could be
551 attributed to their larger size and more mature anatomy (e.g. fused skulls) of these birds (Hogg
552 1982), therefore more extensive trauma may be required to cause rapid loss of reflexes.
553 Furthermore, the pupillary reflex is affected by disruption to the blood supply of the retina (e.g.
554 severing of carotid arteries), therefore observed dilation and constriction of the pupil may not be
555 due to a genuine reflex to the light, and thus the pupillary reflex durations for the NMCD and the
556 MCD may be inadvertently elongated (Bilello *et al* 2003; Gregory & Wotton 1990; Perry *et al*
557 2012; Sharma *et al* 2005). However, it is important to note that more than 75% of all birds across
558 all killing methods showed pupillary reflex in the first 15 s post-application of a kill treatment,
559 suggesting that none of the devices caused immediate brain death.

560

561 The MZIN was associated with significantly shorter jaw tone durations than NMCD or MCD,
562 which has been used as an indicator of consciousness (Croft 1961; Erasmus *et al* 2010a; Erasmus
563 *et al* 2010c), suggesting that MZIN caused birds to lose consciousness faster than the other two
564 killing methods, when successful. In broilers, NMCD resulted in shorter jaw tone durations
565 compared to MCD and there was a significant effect of bird age (which was confounded with bird
566 type, as all broilers were less than 5 weeks of age, despite being heavier than mature layer hens).
567 This could be explained by the fact that late production broilers and mature layer hens were heavier
568 birds and therefore have a greater volume of blood and larger blood vessels, which could make it
569 more difficult to stop or minimise blood flow to the brain stem, which controls jaw tone (Solomon,
570 1990; Whittow, 2000). MCD and NMCD did cause sufficient damage to the brain stem across all

571 birds, demonstrated by short mean durations for jaw tone, as well as less than 40% of birds ever
572 showing the reflex. Sandercock and colleagues (2014) showed that unconsciousness induced by
573 anesthetic was associated with loss of jaw tone in layers and turkeys and was a consistent measure
574 of loss of consciousness in this context. For birds which did not lose jaw tone immediately post
575 device application, there is concern that the birds may be conscious, however the absence of other
576 reflexes alongside (e.g. nictitating membrane and rhythmic breathing) would suggest this may not
577 be the case, and the presence of jaw tone may be indicative of damage to the larynx (Cors *et al*
578 2015; Silvano *et al* 1996), which can result in spontaneous “gagging” or perceived “gasping”
579 behaviours and resulting in perceived jaw tone. These behaviours are not indicative of
580 consciousness and are present in the absence of auditory evoked potentials (Cors *et al* 2015).

581

582 The ceasing of clonic death-related behaviours (e.g. leg paddling and wing flapping) has been used
583 as an indicator of time of death for poultry which are killed by CO₂ gas stunning (Gerritzen *et al*
584 2007), and based on this, all three killing methods were shown to kill birds in similar time periods,
585 despite small differences attributed to bird type and age, which may be indicative of variation in
586 bird nutrition and available muscle glycogen (Debut *et al* 2015; Petracci *et al* 2010). The majority
587 of birds showed convulsive wing flapping and leg paddling, which has been observed in several
588 other studies of killing with various methods (Abeyesinghe *et al* 2007; Lambooi *et al* 1999;
589 McKeegan *et al* 2007). The onset of cloacal movement, where visible, was the last reflex observed
590 before all movements ceased, which may highlight it as a conservative indicator of death.

591

592 **Conclusion and Animal Welfare Implications**

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

605

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607

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