HUMANE MECHANICAL METHODS FOR KILLING POULTRY ON-FARM

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By Jessica E Martin, B.Sc (Hons), M.Sc.

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Abstract

Worldwide, an estimated 9.1 billion birds may need to be killed on farm each year. As of January 2013 the use of manual cervical dislocation (MCD) as a killing method for poultry on-farm has been heavily restricted through new EU legislation (EC 1099/2009) on the Welfare of Animals at the Time of Killing, following reported welfare concerns. The method by which birds are killed on farm is crucial to poultry welfare on a large scale. The overall aim of this project was to design a mechanical device conforming to the new legislation to kill poultry humanely on-farm and provide a competitive replacement for MCD.

Following a survey and a literature review, four mechanical devices were designed and prototyped: Modified Armadillo (MARM); Modified Pliers (MPLI); Modified Rabbit Zinger (MZIN) and a Novel mechanical cervical dislocation glove (NMCD). The devices were tested for killing efficacy in three laboratory experiments, assessing their performance in poultry cadavers (Study 1), anaesthetised birds (Study 2) and live conscious birds (Study 3). The reliability and welfare impact of the devices, along with comparisons with a control method (MCD) were evaluated via post-mortem analysis, reflex and behaviour durations, and characteristics of electroencephalography (EEG) analysis. Due to consistently high kill success rates and rapid loss of reflexes, as well as short durations of EEG activity indicating consciousness across three laboratory experiments, the NMCD device was shown to have the most promise as a mechanical device to be used as an alternative to MCD for poultry stock-workers and keepers. The final experiment explored the user-reliability and practicality of the NMCD device in two relevant commercial environments (a layer hen farm and a broiler farm). When applied by multiple users, the NMCD device did not match the killing performance of MCD, however it did show promise and the study highlighted the need for further refinement in the training protocol in order to encompass the wide variation in MCD techniques and experience.

The result of this project is a novel on-farm mechanical killing device, which shows great potential in laboratory experiments and competed with the traditional MCD method in commercial environments. Further training refinements are required in order to develop the device into a marketable product which any individual could purchase and use as a humane method for killing poultry.

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Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature:

Printed name: Jessica Martin

List of Abbreviations

CNS	Central nervous system
Df	degrees of freedom
ECG	Electrocardiogram
ECI	Electrocerebral inactivity
EEG	Electroencephalogram
ERs	Evoked responses
F50	Median frequency
F95	Spectral edge frequency
GLMM	Generalised Linear Mixed Models
ISO	Isoelectric
JT	Jaw tone
LP	Leg paddling
MARM	Modified Armadillo
MCD	Manual cervical dislocation
MPLI	Modified Pliers
MZIN	Modified Zinger
N	Sample size
NIC	Nictitating membrane reflex
NMCD	Novel mechanical cervical dislocation
PTOT	Total power
PUP	Pupillary (light response) reflex
RB	Rhythmic breathing
SD	Standard deviation (±)
SE	Standard error (±)
VW	Cloacal movement
WF	Wing flapping

1 Background

1.1 Introduction

The humane killing of livestock on-farm is a fundamental part of successful livestock management. Small numbers of livestock may need to be killed on-farm for four main reasons: (1) to prevent suffering from injury or sickness; (2) disease control management; (3) livestock management (e.g. male layer stock); and (4) small 'farm-gate' sales of animal products. The on-farm killing of animals, also occasionally referred to as culling, does not include the killing of animals for slaughter. The EU Regulations on the welfare of animals at the time of killing defines slaughter as "causing the death of the animal by bleeding", while the killing of animals is defined as "causing the death of the animal by any process other than slaughter" (European Council, 2009).

Approximately 50 billion chickens are reared annually worldwide and, with average mortality rates from hatching to pre-slaughter stages ranging from 1-15% in the main chicken producing countries, it can be estimated that 500 million to 7.5 billion chickens may need to be killed on-farm each year. In the United Kingdom (UK) alone, It was estimated that 46 million birds (broilers and layers) died or were despatched on-farm in 2009 (DEFRA, 2010). Therefore the methods utilised for killing chickens on-farm are important to secure the welfare of these animals. Routine slaughter methods for poultry have been thoroughly researched and since the end of the 19th century they have become highly mechanised (e.g. shackling, electrical stunning, stunning by captive bolt and mechanical exsanguination (Fletcher, 1999; Gregory, 2005; Hindle et al., 2012; Petracci et al., 2010; Petracci et al., 2006; Raj et al., 1998; Scott, 1993). However, on-farm killing methods have not received the same scientific scrutiny until the last few decades.

Methods for killing poultry can be split into two categories; manual and mechanical. For this review, the definitions of manual and mechanical killing methods are separated by the simple distinction that a manual method only involves the use of the operator's body (e.g. hands), while a mechanical method is defined as the use of any mechanical device or aid which enhances the operator's ability to kill the bird. This categorisation has been discussed and agreed with DEFRA and the Humane Slaughter Association (HSA) (personal communication) and can be easily implemented within the current European Regulations (European Council, 2009). The current industry standard for killing poultry on-farm is manual cervical dislocation (i.e. ‘necking’ by hand). However, some turkey producers are an exception, as they use mechanical methods to dispatch birds on-farm (e.g. pliers or concussive devices), partly due to the larger size (and weight) of the birds, but also to comply with sort after food assurance schemes, like Quality British Turkey and Freedom Foods (RSPCA, 2010).

Over the past few decades, animal welfare has risen in priority in society due to scientific research exploring the phenomenon of animal consciousness and sentience, as well as increased public interest in our relationships with animals and the resulting public perception of animal welfare. As a result, the majority of standard livestock management procedures (e.g. castration, tail docking, beak trimming, etc.) have started to receive increased scientific attention in their relation to welfare. As part of this ‘welfare movement’, scientific research has started to question the humaneness of on-farm killing methods, in particular manual cervical dislocation (MCD) in poultry (Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory and Wotton, 1990) and its suitability across all poultry species and at different developmental stages, particularly in terms of its consistency and the issue of stock-worker fatigue (Gregory and Wotton, 1990; HSA, 2002; HSA, 2004). This uncertainty in the humaneness of manual cervical dislocation has led to various organisations questioning its use and in some cases restricting it.

In 2013, the new EU regulations on the killing or slaughtering of animals came into effect (European Council, 1993; European Council, 2009). These new regulations have noted the welfare concerns associated with some on-farm killing methods and have restricted their use accordingly. The use of MCD in poultry will be limited to ‘emergency killing’, which does not require prior stunning, but cannot be used on birds which weigh over 3 kg (European Council, 2009). The number of birds which an operator can kill with this method in one day is also restricted, to 70 birds per person per day (European Council, 2009). These weight and number limits have not apparently been justified through scientific research, and it is difficult to identify where they originated, although the 3 kg weight limit for killing poultry by manual cervical dislocation was also stated in the EU Regulations on the protection of animals used for scientific purposes (European Council, 1986). However, it is logical to suggest that the number limit could be perceived as high enough to allow for dispatching casualty birds found on daily inspections on-farm, but low enough to prevent the method from being used as a routine procedure to kill birds (e.g. killing a shed of end-of-lay hens). The weight limit, however, in reality does not practically affect the poultry industry as on the whole broilers and laying hens do not exceed 3 kg before they are killed or slaughtered, and birds which do exceed the weight limit (e.g. turkeys) tend to be killed by other methods. Therefore, it could be perceived that the legislation is attempting to reduce the use of a method which may or may not be humane, but not completely prevent its use as this is likely to be met with protest by the poultry industry. The EU Regulations (European Council, 1986) on use of animals for scientific purposes has also been recently updated and amended (European Council, 2010), which restricts the use of manual cervical dislocation even further to birds weighing less than 1 kg, and birds which weigh over 250 g should be sedated prior to application if appropriate (European Council, 2010). The new EU Regulations on welfare at killing (European Council, 2009) has also restricted another poultry killing method: neck crushing methods (e.g. Semark Pliers) are not mentioned in the regulations, and thus by their omission are not permitted. These have been deemed inhumane and should not be used (HSA, 2002; HSA, 2004).

FAWC (2009) recommended research to explore current and novel methods for killing poultry in small numbers. Several mechanical devices have been developed recently (e.g. CASH Poultry Killer, Turkey Euthanasia Device) (Erasmus et al., 2010a; Erasmus et al., 2010b; HSA, 2004; Raj and O'Callaghan, 2001); however, none have been enthusiastically adopted by the commercial industry or small poultry keepers due to issues of cost, maintenance and practicality. However, mechanical cervical dislocation is less restricted by EU regulations (European Council, 2009) than manual dislocation and could minimise the issues which manual dislocation is suggested to suffer from (e.g. inconsistency and operator fatigue) (Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory and Wotton, 1990; HSA, 2004).

The aim of this review is to evaluate the humaneness and physiological effects of the current poultry killing methods in order to identify possible avenues for future research and development of new and improved mechanical killing methods which will improve welfare at killing on farms as well as complying with the EU regulation (1099/2009) on the Protection of Animals at the Time of Killing (European Council, 2009).

1.2 Defining death and unconsciousness

Death can be easily defined as the ending of an animal's life. However, if one then attempts to define life, it soon becomes clear that the two are highly interlinked and there are still many aspects of both which are not understood, resulting in little consensus. This is illustrated by the fact that several different organisational bodies (e.g. Human Law, Medicine, Scientific Research) all currently produce their own definitions. The definition of death has become even more difficult to untangle as medical and scientific research have advanced over the last century (e.g. with the advent of resuscitation and life support). However, it is currently accepted that death is not a single event, but a process in which an animal transitions from being alive, through varying levels of consciousness to eventual death (Baron et al., 2006; Gordon, 1944). A new concept for describing and defining death was introduced in the late 1950s (Mollaret and Goulon, 1959) and was termed "brain death". This term was then elaborated and became known as the "Harvard criteria", which detailed the diagnosis of brain death as the absence of cerebral responsiveness, spontaneous and reactive behaviours, rhythmic breathing, and brain stem (cranial) reflexes (Anon, 1968; Anon, 1977; Knudsen, 2005).

For livestock and other domestic animals, the definition of death is limited to cessation of respiration and cardiac activity, although some consideration is given to "methods of euthanasia when determining the criteria for confirming death" (AVMA, 2007). The main defined states of death in animals are cessation of brain function, cardiac arrest, cessation of breathing and cessation of blood circulation (Baron et al., 2006; European Council, 2009; HMSO, 1995; Widjicks, 1995; Widjicks, 2002), all of which are linked to the diagnosis of brain death (Anon, 1968; Anon, 1977; Knudsen, 2005). As a result, all killing and slaughter methods for livestock and domestic species have been developed around targeting one or more of these causes of death. For example, punctilla killing methods attempt to sever the brain stem (death by cessation of brain function) (Blackmore et al., 1995; Dembo, 1894; Limon et al., 2009; Limon et al., 2010; Limon et al., 2012); electrical stun-to-kill methods cause death by cardiac arrest (Anil, 1991; Anil et al., 1998; Beyssen et al., 2004; Gregory and Wotton, 1985; Gregory and Wotton, 1994); and decapitation (and cervical dislocation) causes death by cerebral ischemia (e.g. restriction of blood supply to the brain) (Bates, 2010; Carbone et al., 2012; Cartner et al., 2007; Erasmus et al., 2010a; Gregory and Wotton, 1990; McNeal et al., 2003; Van Rijn et al., 2011).

Research in mammalian physiology has demonstrated that the brain is fundamental in sustaining life (Baron et al., 2006; Rosenberg, 2009; Solomon, 1990; Widjicks, 1995; Widjicks, 2002). The structure of the mammalian brain has been extensively studied and the functions of localised areas have been determined. Possibly the most important of these areas in terms of killing has been identified as the brain stem (Gordon, 1944; Kendrick, 2007; Solomon, 1990; Whittow, 2000). The brain stem is located at the back of the brain and joins onto the spinal cord, although there is no defined structural segregation between them (Günther and Necker, 1995; Solomon, 1990; Whittow, 2000). The brain stem is composed of three areas; the medulla oblongata, the pons, and the midbrain. The brain stem has many vital functions, such as respiratory and cardiovascular control, however its main role is the conduction of information (action potentials) through ascending and descending pathways from the rest of the body to the cerebrum and the cerebellum in the brain (Günther and Necker, 1995; Solomon, 1990; Whittow, 2000). Therefore, it is understandable that the majority of killing methods are focused around destroying the brain as a whole or severing it from the body (i.e. destruction of the brain stem), as this would result in all the defined causes of death being likely to occur.

However, the other important factor affecting how to kill an animal is its welfare, therefore providing a ‘humane death’ is essential (AVMA, 2007; European Council, 1993; European Council, 2009; HMSO, 1995; HSA, 2002). The issue of animal sentience (and/or consciousness) has been thoroughly debated since the late 20th century (Boissy et al., 2007; Broom, 2007) and it is now widely accepted that vertebrate animals (especially mammals and birds) are sentient beings that can experience emotions (including pain) and may have some concept of self-awareness (Anil et al., 2002b; Bateson, 1991; Beshkar, 2008; Boissy et al., 2007; Broom, 2007; Gentle, 2011). As a result, concepts of animal welfare have evolved to become more complex (e.g. The Five Freedoms (FAWC, 2007)), and represent more than just providing the basic physiological needs for an animal (e.g. food and water). Animal welfare is now considered to also encompass the animal’s psychological needs and prevention from experiencing unnecessary negative emotions (Broom, 2007).

Consciousness is an elusive term, which has been broadly and variously defined in an attempt to classify the highly subjective phenomenon of emotions ('feelings') and self-awareness (Boissy et al., 2007; Broom, 2007; Kendrick, 2007). The existence of human consciousness has been accepted without question, due to our ability to communicate to one another that we experience emotions and that we are aware of ourselves. As a result it is prudent to assume that if one human perceives himself or herself as a conscious being, then other human individuals are likely to be conscious also (Philips 2008, Boissy et al 2007, Broom 2007). However, if consciousness is determined by self-report, then questioning whether other animals are conscious becomes an impossible task due to our inability to communicate across species. Following Darwin's theory of natural selection, consciousness must have some adaptive value, which has evolved over time. However identifying where consciousness began in the evolutionary history of animals is currently an impossible task. There is considerable scientific evidence (e.g. behavioural plasticity, mirror self-recognition, intra-species communication etc.) which supports the notion that animals may have some form of sentience at varying levels of complexity (Beshkar, 2008; Griffin and Speck, 2004; Morin, 2006; Rosenberg, 2009). In this thesis, consciousness is defined following the theory of medical awareness and "state-consciousness" (De Graaf et al., 2012; Hohwy, 2009), where consciousness is evaluated in various states e.g. sleep/awake (Corner et al., 1973; Massimini et al., 2005; Rattenborg et al., 2009; Sandercock et al., 2014), healthy/comatose (Dunham et al., 2012; Owen et al., 2006; Rosenberg, 2009), and drugged/sober animals (Ferrarelli et al., 2010). This is separate to the term sentience which has been used to define cognitive abilities and the capacity to perceive positive and negative mental states (Beshkar, 2008; De Graaf et al., 2012; Duncan, 2006; Griffin and Speck, 2004; Morin, 2006).

In the case of birds, there is considerable scientific evidence to suggest that birds exhibit conscious states much like mammals (Corner et al., 1973; Rattenborg et al., 2009) and are sentient (Gentle, 2011; Gentle and Tilston, 2000; Rutherford, 2002), with the complexity being species dependent. Evidence based on avian brain physiology demonstrates that, like the mammalian brain, the avian brain has homologous structures to suggest sentience like that suggested in mammals (e.g. the Wulst and the anterior dorsal ventricular ridge Butler et al., 2005). There are also similarities in the electrical activity patterns of the brain in awake birds compared to mammals; however the patterns do differ between the two for sleep states (Edelman et al., 2005).

In terms of killing animals, the most important concern that arises in terms of welfare is the ability of the animal to experience pain and other negative states, including physiological distress (e.g. respiratory distress) during the process. Pain is described as having both physical (sensory) and psychological (motivational and emotional) aspects in response to potential or genuine tissue damage (Anil et al., 2002b; Bateson, 1991; Bennett, 1997; Gentle, 2011; Guatteo et al., 2012; IASP, 1979; Rutherford, 2002). By contrast, nociception specifically refers to the sensory ability of an animal to perceive noxious stimuli (e.g. specific ranges of mechanical, chemical and temperature stimuli Anil et al., 2002b; IASP, 1979; Wiech et al., 2008). However, demonstrating that an animal experiences pain is much more difficult due to its emotional component and subjective nature (Anil et al., 2002b; Bateson, 1991; Bennett, 1997; Gentle, 2011; Rutherford, 2002). In humans, pain can only be confirmed by self-report by the individual experiencing the sensation, therefore as animals cannot directly communicate, pain can only be inferred by indirect measures, for example, behaviour (e.g. licking an injury site) or physiological variables (e.g. elevated heart rate) (Arras et al., 2007; Gentle, 2011; Gentle and Tilston, 2000; Rutherford, 2002). Following Darwin’s theory of natural selection, pain must have evolved and been maintained in animals because it provided some advantage. Several scientists have suggested that pain has considerable protective functions, as it discourages an animal from repeating the encounter with the potentially (or genuinely) noxious stimuli, as well as protecting the injury from further damage by discouraging behaviour which aggravates it (Gentle, 2011; IASP, 1979; Rutherford, 2002; Wiech et al., 2008). However, in the case of poultry, a prey species, it is in the animal’s interest of survival to mask any sign of illness or injury, such as behaviours indicating pain, to protect against predation. Therefore it is logical to suggest that if a chicken does display pain related behaviour (Bateson, 1991; Gentle, 2011; Gentle and Tilston, 2000; Rutherford, 2002), the level of pain it may actually be experiencing could be a very significant welfare issue.

It is important to note that humane killing of livestock animals for slaughter or on-farm killing should not be confused with the term euthanasia. Euthanasia is defined literally as providing a ‘good death’ or providing a death that is in the animal’s interest and this is put into practice as killing an animal as painlessly as possible or by allowing it to die by withholding veterinary intervention (Bates, 2010). However, it is only applicable to sick or injured animals (veterinary patients) or animals used in scientific research (AVMA, 2007; Bates, 2010). Therefore only animals killed on-farm for welfare reasons applicable to sickness or injury can be termed as euthanasia, but this is only one of the four main reasons for killing animals on-farm. Euthanasia is therefore often a misused term for describing killing of animals on-farm. During slaughter, all animals in the UK and the rest of the EU must be stunned prior to exsanguination by law (e.g. electrical stunning or captive bolt) to render them unconscious (religious slaughter is exempt from this requirement - Barnett et al., 2007; European Council, 2009; HMSO, 1995). Emergency killing methods, however, do not have to include a pre-kill stun step (HSMO 1995). Therefore the animals will be conscious (if not rendered unconscious from sickness or injury) when the killing technique is applied and will potentially be able to experience negative emotions (e.g. pain and stress), if the killing method does not render them immediately unconscious. However, it is important to note that during the process of stunning the animal, it is likely the animal may feel stressed due to handling and restraint (Gregory and Wotton, 1994; Jones, 1996; Petracci et al., 2010; Schilling et al., 2008; Scott, 1993) and the scientific community are still unable to definitely conclude that the animal will not feel pain when the stunning device is applied, albeit over a relatively short period of time. There is also the consideration that a number of stunning methods do not result in instantaneous loss of consciousness, such as gas stunning (Lambooij et al., 1999a; McKeegan et al., 2013a; Poole and Fletcher, 1995; Raj, 1999; Raj et al., 1998; Sandilands et al., 2006a; Sandilands et al., 2006b; Webster and Fletcher, 2004).

Another commonly used term for killing an animal is culling. Literally defined, culling is described as identifying and removing individuals/objects from a group, for example, selecting individual cows from a herd for sale or to be killed (Fetrow et al., 2006). The term culling is not limited to killing animals for non-slaughter reasons, but for permanent removal of an animal for any reason. However, its use is highly varied and species specific, for example in poultry, culling is more commonly used when referring to killing end-of-lay hens or ‘spent’ broiler breeders or killing healthy animals within a ‘risk area’ during a disease outbreak (Raj et al., 2006a), while in cattle it refers to only the removal (not necessarily the killing) of low-milk yield cows (Fetrow et al., 2006; Langford, 2012). In the case of poultry, all published uses of the term culling refer to birds being killed as a result of the cull (i.e. not including live sales), and the majority of authors use culling when referring to killing for disease outbreak management (Lund et al., 2000; Raj, 2006; Raj et al., 2006a). It could be argued that the use of the term 'culling' is a slightly more ambiguous term in comparison to 'killing'. Management of notifiable disease outbreaks in all livestock species usually involves the killing of diseased animals as well as healthy animals which are within a specific distance to the infected farm, in an attempt to isolate the outbreak (Lund et al., 2000; Raj, 2006; Raj et al., 2006a). Therefore, when reporting the killing of large numbers of animals, some of which are healthy, it may be more appealing for the writer and less aversive for the reader to use the term 'culling' rather than 'killing'.

1.2.1 Methods for evaluating death and unconsciousness in livestock

When using the Harvard criteria to define brain death of animal, methods of evaluating the set criteria must be available. One of the key methods for assessing brain function (including brain death and conscious state) is the use of the electroencephalogram (EEG), which is a recording (via electrodes placed on the scalp or on the surface of the brain (i.e. dura)) of the spontaneous electrical potentials produced by cells within the cerebral cortex (Anon, 1968; Anon, 1977; Firsching et al., 1992; Knudsen, 2005; Lowe et al., 2007; Pallis and MacGillivray, 1980). States of consciousness and brain death are assessed by changes to the "normal" or baseline EEG pattern, observed in a conscious animal (Pallis and MacGillivray, 1980; Sandercock et al., 2014). For example, sleep or anaesthesia are characterised by high amplitude slow waves (Baars et al., 2003; Sandercock et al., 2014). Conscious states (i.e. information processing) are associated with high frequency, low amplitude waveforms, which can have unsynchronised patterns (Baars et al., 2003; Simons et al., 1989). Changes or abnormalities in the EEG pattern are represented by distortion of the pattern, as well as an increase in the distortion, and finally electrocerebral inactivity (ECI), also termed isoelectric EEG, where the signal is a flat line, which indicates no brain function and therefore brain death (Buchner and Schuchardt, 1990; Dunham et al., 2012; Facco et al., 2002; Firsching et al., 1992; Grigg et al., 1987; Machado, 2004; Pallis and MacGillivray, 1980). However, care has to be taken when using EEG to confirm brain death, as the electrodes only record the activity in the cerebral cortex. Deeper areas in the brain and sites responsible for basic functions (e.g. respiration and cardiac activity), for example the brain stem, may still be functioning despite extensive trauma to the cerebral cortex, resulting in a comatose state in the animal (Buchner and Schuchardt, 1990; Firsching et al., 1992; Grigg et al., 1987; Machado, 2004; Pallis and MacGillivray, 1980; Widjicks, 2002). Likewise, the presence of EEG activity may not always suggest consciousness or brain function, e.g. the EEG signal is highly susceptible to electrical noise artefact, which contaminates the signal, preventing a complete flatline when isoelectric (Delorme and Makeig, 2004; Gwin et al., 2010; Lowe et al., 2007). Derived measures from the EEG include evoked responses (ERs), which are the analysis of the electrical responses to repeated visual, auditory or somatosensory stimuli compared to the ongoing spontaneous EEG signal (Gregory and Wotton, 1990; Knudsen, 2005). However several studies have demonstrated the weaknesses of ERs as a diagnosis of brain death as they are time consuming to collect and they are poorly correlated with other brain death measures (Facco et al., 2002; Firsching et al., 1992; Pallis and MacGillivray, 1980). It has even been suggested that ERs are a measure of sensory processing rather than consciousness (DEFRA, 2014).

An inconsistency with the use of EEG recordings is scientists' use of the term "EEG activity". Some researchers will state an observed reduction in EEG activity (e.g. absence of ERs), while others will state that EEG activity becomes isoelectric. The EEG activity pattern of the chicken has been documented in awake/sleep states (Table 1.1) and induced unconscious states via anaesthetics (Sandercock et al., 2014). It has been suggested that for a bird to be unconscious it must have an EEG output, i.e. total power (PTOT) and median frequency (F50), of less than the average sleep range (Prinz et al., 2012; Van Rijn et al., 2011). A study conducted by van Rijn and colleagues (2011) used this argument to explore the time it takes for rats to become unconscious post-decapitation and their results showed that on average it took 17 seconds for EEG output to become isoelectric. When an EEG output becomes isoelectric, one can confidently suggest there is no electrical brain activity and brain death has occurred (Machado, 2004; Prinz et al., 2012; Van Rijn et al., 2011). The majority of papers investigating EEG output in relation to on-farm killing methods do not define what they consider to be a reduction in EEG activity. Some authors have suggested that a sufficient reduction in EEG output has to be a reduction to at least 10% of baseline (awake) power (based on EEG power spectrum analysis) in order to infer unconsciousness (Gregory and Wotton, 1990; Prinz et al., 2012; Tidswell et al., 1987). However, some studies merely suggest that any reduction in EEG activity (e.g. loss of ERs) can be inferred as a change in ‘mental state’ and the probability of consciousness (Holson, 1992; Mikeska and Klemm, 1975). However, since EEG output has been shown to be highly species specific and subject to individual variation, several studies dispute this (Erasmus et al., 2010a; Gregory and Wotton, 1990; Van Rijn et al., 2011).

Table 1.1 Documented EEG output for adult laying hens (Ookawa, 1972; Rattenborg et al., 2009)

Bird state	EEG output	
	Power	**Wave frequency**
Awake (excited)	~20-50µV	~30-60Hz
Awake (unexcited)	~50-150µV	~17-24Hz
Sleep (rest – stage 1)	~50-150µV + irregular bursts of ~200-300 µV	~17-24Hz + irregular bursts of ~3-12Hz
Sleep (true sleep – stage 2)	~200-400µV	~6-12Hz + irregular bursts of ~3-4Hz

A more powerful analysis technique for EEG output is now being used, which transforms sections of the original EEG trace, via Fast Fourier Transform (FFT) analysis, into power frequency spectra in other words, graphical summaries of the relationship between the frequency and power of the EEG waveform (Coenen et al., 2009; Delorme and Makeig, 2004; McKeegan et al., 2013a)). From this, spectral variables (derived from the area under the frequency spectrum graph) can be used to generate variable ranges for distinct consciousness states. Examples of these spectral variables are: total power (PTOT) – the total area under the frequency spectrum; Median frequency (F50) - the frequency below which 50% of the EEG power resides, and spectral edge frequency (F95) - the frequency below which 95% of the EEG power resides (Figure 1.1) (Johnson et al., 2005a; McKeegan et al., 2007; Murrell and Johnson, 2006; Murrell et al., 2008; Sandercock et al., 2014; Tonner, 2006). The use of this analysis has been well documented and has allowed detailed evaluation of EEG activity (Becker et al., 2010; Delorme and Makeig, 2004; Gwin et al., 2010; Johnson et al., 2005a; McKeegan et al., 2013a; McKeegan et al., 2013b; Sandercock et al., 2014). Changes in EEG activity resulting from a bird going from a conscious to an unconscious state are indicated by a decreasing F50 and a sharp increase in PTOT (McKeegan et al., 2013a; McKeegan et al., 2013b; Sandercock et al., 2014) (Table 1.2).

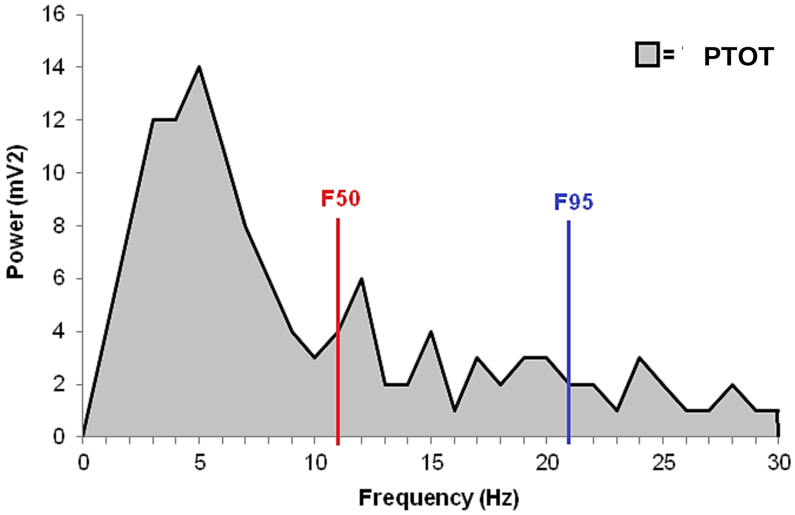


Figure 1.1 A representative example of a frequency spectrum from a 2 s EEG epoch, demonstrating the derived values for total power (PTOT), median frequency (F50), and the spectral edge (F95).

Table 1.2 Documented mean spectral variables (± SD) calculated from 2 s EEG epochs in layer hens for awake, unconsciousness and brain dead states (Sandercock et al., 2014). PTOT = total power, F50 – median frequency, F95 = spectral edge.

Spectral variable	Conscious state		
	Awake (fully conscious)	**General anaesthesia (unconscious)**	**Brain dead (i.e. ECI/isoelectric)**
PTOT (μV^2)	1592 ± 501	13413 ± 4238	194 ± 46
F50 (Hz)	24 ± 5	7 ± 2	46 ± 4
F95 (Hz)	82 ± 7	26 ± 6	91 ± 3

Another concern with the use of EEG activity as an indicator of brain death and conscious state is its high sensitivity to recording artefacts, such as background electrical noise or "mains hum" at ~50 Hz, animal movements, eye spindles, etc., which have been thoroughly documented (Delorme and Makeig, 2004; Gwin et al., 2010; Lowe et al., 2007; McKeegan et al., 2013b). There is also an issue with substantial individual variation, which has been reported to affect diagnostic certainty by up to 20% (Buchner and Schuchardt, 1990). As a result, it is recommended that combined methods for evaluating brain death be used, in order to improve the reliability of diagnosis (Buchner and Schuchardt, 1990; Facco et al., 2002; Firsching et al., 1992). In terms of on-farm killing as well as animal slaughter, the methods used to assess loss of consciousness and brain death must be simple and practical to use in the field. Recording EEG activity is not possible in a commercial/on-farm situation (Erasmus et al., 2010c; Knudsen, 2005). Therefore assessment is made using the loss of reflexes/behaviours, cessation of respiration and cessation of cardiac activity, which infer states of consciousness and/or eventual brain death. The choice of which reflexes and behaviours to use can be killing method and species dependent (Anil, 1991; Anil et al., 1998; Croft, 1961; Erasmus et al., 2010c; Prinz et al., 2012; Sandercock et al., 2014; Shaw, 1989; Verhoeven et al., 2014). For poultry as well as other livestock species, the complete loss of all brain stem reflexes and behaviours is an indicator of brain death, and therefore complete insensibility and unconsciousness (Anon, 1968; Erasmus et al., 2010c; Heard, 2000; Sandercock et al., 2014; Verhoeven et al., 2014; Widjicks, 2002). The common reflexes and behaviours seen during loss of consciousness and dying are listed in Table 1.3. However, individual interpretation of each reflex or behaviour has been shown to not be reliable for indicating brain death or unconsciousness (Anil, 1991; Anil et al., 1998; Blackmore, 1984; Erasmus et al., 2010c; Knudsen, 2005; Rosenberg, 2009; Sandercock et al., 2014; Shaw, 1989; Widjicks, 1995; Widjicks, 2002). For example, animals killed by captive bolt show loss of posture, rhythmic breathing, and corneal reflex, but spinal reflexes are inadequate due to the convulsive and erratic nature (Finnie et al., 2000; Finnie et al., 2002; Gregory et al., 2009).

Table 1.3 Reflex and behavioural indicators for assessing unconsciousness and brain death in livestock for on-farm killing methods.

Reflex/ Behaviour	Neurological control area *	Description	Indicator of unconsciousness / brain death +	Issues of use +
Pupillary (light) reflex	Brain stem and cranial nerves	Constriction reaction of the pupil to light directed into the eye.	Brain death+	• Difficult to assess in damaged eyes (i.e. as a result of captive bolt). • Difficult to assess in brightly lit surroundings.
Nictitating membrane reflex	Brain stem and cranial nerves	Membrane closes over eye in response to mechanical touch stimulation of the medial canthus.	Brain death+	• Difficult to assess in damaged eyes (i.e. as a result of captive bolt).
Palpebral reflex	Brain stem and cranial nerves	Blinking of the eyelid in response to tapping the edge of the eye and eyelid.	Unconsciousness	• Difficult to assess in damaged eyes (i.e. as a result of captive bolt).
Jaw/neck tone	Brain stem and cranial nerves	Resistance to downward manipulation and pressure applied to the lower beak/jaw and tension in the neck, ability to hold head upright.	Unconsciousness	• Jaw tone can be misdiagnosed as gasping or gaping behaviours. • Difficult to assess neck tone in cervical dislocation methods as trauma to the neck muscle prevents this behaviour.
Cloacal movement	Brain stem and cranial nerves	Sporadic opening and closing of the cloaca sphincter.	Brain death	• Difficult to assess when other clonic behaviours occur.
Cardiac activity	Brain stem	Activity of the heart (resting heart rate to no heart beat).	Brain death	• Equipment required (e.g. stethoscope, Doppler). • Difficult to assess when other clonic behaviours occur, bird needs to be restrained.
Rhythmic breathing	Brain stem	Rhythmic respiration (inhalation and exhalation).	Brain death	• Difficult to assess when other clonic behaviours occur, can be easily misdiagnosed.
Wing flapping	Spinal cord effectors	Clonic flapping of the wings in a sporadic fashion.	Brain death	• Experience required in identifying the convulsive behaviour in order to prevent genuine voluntary movements (and ineffective kill) being missed.
Leg paddling	Spinal cord effectors	Clonic movement of the legs in a sporadic fashion.	Brain death	• Experience required in identifying the convulsive behaviour in order to prevent genuine voluntary movements (and ineffective kill) being missed.
Pedal reflex	Spinal cord effectors	Swift retraction of foot in response to a hard pinch applied to a toe. Demonstrates a positive reaction to painful stimulus.	Unconsciousness	• Paralysis of the animal as a result of trauma prevents correct assessment of reflex.

** (Erasmus et al., 2010c; Knudsen, 2005; Van de Sluis et al., 2009; Whittow, 2000)*

+ (Anil, 1991; Anil et al., 1998; AVMA, 2007; Coles, 1997; Croft, 1961; Erasmus et al., 2010c; Finnie et al., 2000; Gregory et al., 2009; Heard, 2000; Prinz et al., 2012; Raj and Gregory, 1990; Sandercock et al., 2014; Shaw, 1989; Van de Sluis et al., 2009; Verhoeven et al., 2014)

Research evaluating the correlation between durations of EEG activity and reflexes/behaviours is incomplete and difficult to compare across species and different killing methods (Gregory et al., 2007; Gregory et al., 2009; Gregory and Wotton, 1990; Gregory and Wotton, 1994; McKeegan et al., 2013a; McKeegan et al., 2013b; Mikeska and Klemm, 1975; Nicolaou et al., 2012; Pallis and MacGillivray, 1980; Sandercock et al., 2014). Some research has been undertaken in poultry (Gregory and Wotton, 1990; McKeegan et al., 2013a; McKeegan et al., 2013b), however in all cases the EEG activity ceased prior to the loss of any of the cranial or spinal reflexes/behaviours. This suggests that the use of them is a conservative measure of unconsciousness and brain death. For example, Sandercock *et al*. (2014) compiled a list of behaviours and reflexes which were present or absent at various conscious stages induced by sevoflurane anaesthetic and brain death (euthanasia by overdose of barbiturate), Table 1.4 summarises the key results. The majority of reflexes and behaviours are lost when birds are unconscious, however the pupillary and nictitating membrane reflexes persisted, and even once brain death had been confirmed by EEG analysis (isoelectric waveforms) the nictitating membrane was still present, although it was suggested that this may be an artefact of the birds being anaesthetised prior to death (Sandercock et al., 2014).

Table 1.4 Documented presence or absence of reflexes and behaviours in varying states of consciousness in layer hens induced by sevoflurane (general anaesthesia) and overdose of sodium pentobarbital (brain death) (Sandercock et al., 2014).

	Conscious state		
Reflex/behaviours	**Awake (fully conscious)**	**General anaesthesia (unconscious)**	**Brain dead (i.e. ECI/isoelectric)**
Spontaneous righting	present	absent	*
Spontaneous blinking	present	absent	*
Pupillary	present	present	absent
Jaw tone	present	absent	*
Palpebral	present	absent	*
Corneal	present	absent	*
Nictitating membrane	present	present	present

** Not tested – unnecessary following results in unconscious state.*

1.3 Avian anatomy in relation to killing

Death may be caused by the following; cessation of brain function, cardiac arrest, cessation of breathing and cessation of blood circulation (Anon, 1968; Anon, 1977; Baron et al., 2006; Gordon, 1944; Rosenberg, 2009; Widjicks, 2002). All current on-farm killing techniques for small numbers of poultry and game birds either involve (1) the dislocation/severance of the cervical vertebrae, spinal cord and carotid arteries (e.g. necking or decapitation (Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory and Wotton, 1990)); or (2) the destruction of the brain (e.g. blunt force trauma or captive bolt (Erasmus et al., 2010a; Erasmus et al., 2010b; Lambooij et al., 1999b; Raj and O'Callaghan, 2001)). In order to understand how these techniques work and assess their welfare implications, this chapter will focus on the anatomy and physiology of the chicken head (including the brain) and neck.

1.3.1 Avian neck anatomy

The chicken neck is comprised of 14 cervical vertebrae (C1 to C14), which in a rested state are arranged in an 'S' shape (McLeod et al., 1964). The vertebrae are held in place by inter-vertebral cartilage, connective tissue, ligaments, and layers of muscle, with arteries and veins interwoven throughout (McLeod et al., 1964; Whittow, 2000). The cervical vertebrae represent a section in the vertebral column, and each individual vertebra is based around a hollow cylindrical shape, through which the spinal cord runs. The outer surface of the vertebrae is highly varied and defined by articular and transverse processes (Figure 1.2) (McLeod et al., 1964; Whittow, 2000). The two most individual cervical vertebrae are the atlas (C1) and the axis (C2). The ring-shaped atlas is the smallest cervical vertebrae and it attaches to the skull via the occipital condyle (Figure 1.3), to form the occipitoatlantal joint (C0-C1 joint) (McLeod et al., 1964; Whittow, 2000). The axis (C2) attaches the atlas to the rest of the vertebral column. The articulation between the atlas and axis is very minimal, apart from slight ventral movement (McLeod et al., 1964). The remaining cervical vertebrae (C3-C14) tend to have a more uniform, cylindrical shape, with a hollow centre - the vertebral foramen, in which the spinal cord resides. Turkey cervical vertebrae are similar to those of chickens in number as well as structure. However, in ducks and geese the number of cervical vertebrae increases to approximately 16-18 (McLeod et al., 1964).

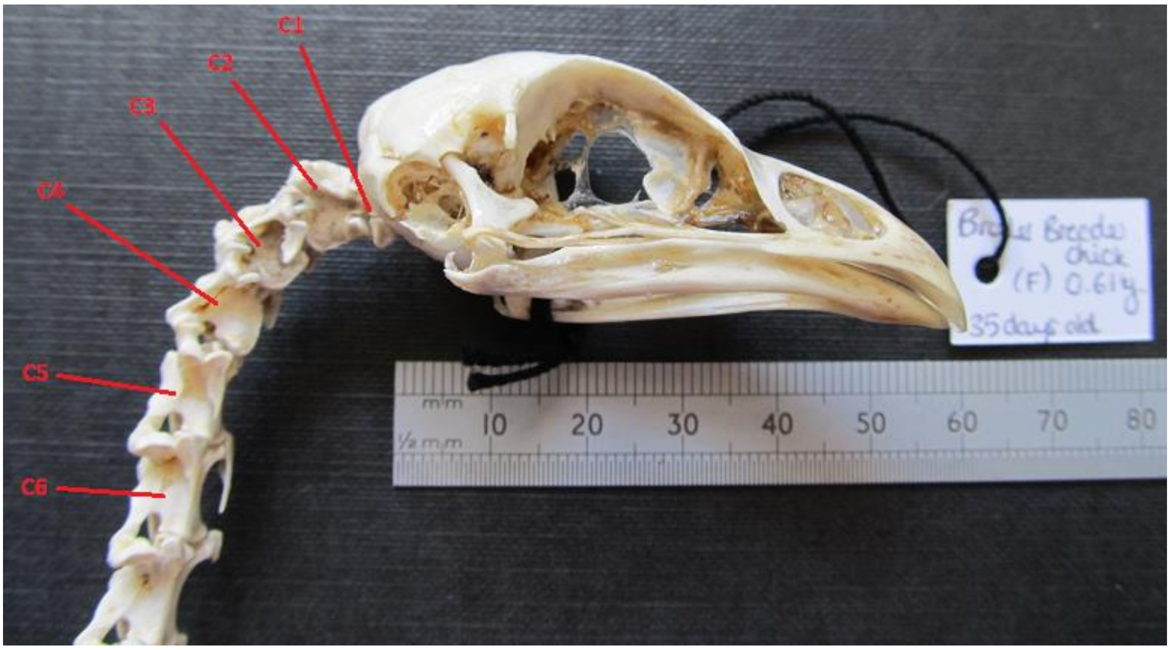


Figure 1.2 Specimen prepared and photographed by the author, demonstrating a chicken skull and the first six cervical vertebrae of a female broiler breeder chick (35 days old).

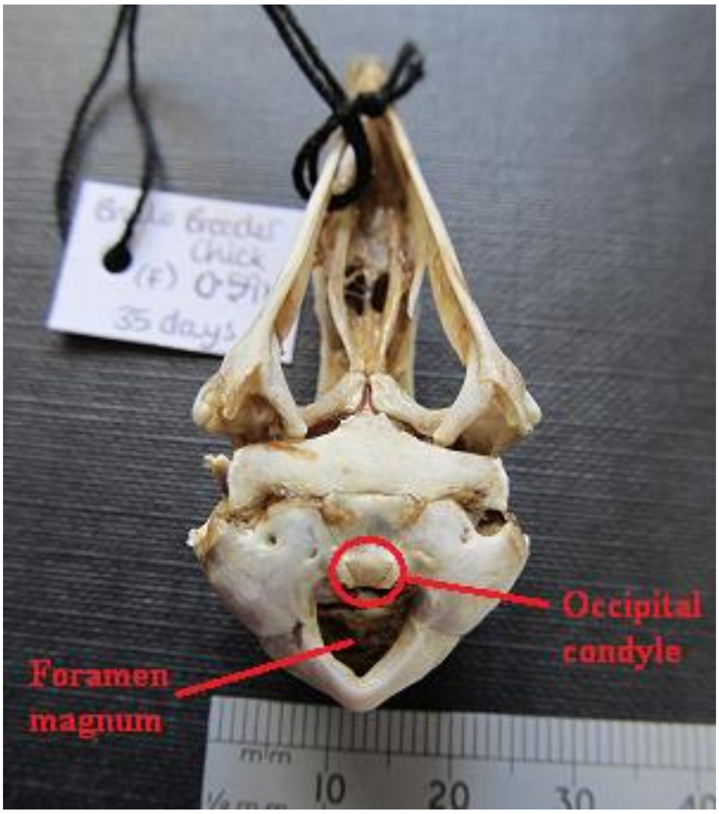


Figure 1.3 Photograph prepared and taken by the author showing the ventral aspect of a chicken skull (female broiler breeder chick, 35 days old). The image highlights the location of the occipital condyle, which is where the occipitoatlantal joint attaches the skull to the atlas. The foramen magnum is the large opening at the back of the skull, through which the spinal cord/brain stem runs, to attach to the base of the brain inside the cranial cavity.

The two primary functions of the cervical vertebrae are to provide support and structure to the neck and head of the animal, and to form a protective structure around the spinal cord (Günther and Necker, 1995; McLeod et al., 1964; Solomon, 1990; Whittow, 2000). However, the vertebral column can be at risk from mechanical trauma in the form of flexion, rotation, compression and extension, which can result in fracture and/or dislocation of the vertebra, and damage to the vertebral column commonly causing damage to the spinal cord (Holdsworth, 1962; Parent et al., 1992; Taneichi et al., 2005; Veras et al., 2000).

The spinal cord is a tubular structure composed of an outer layer of myelinated nerve tracts (white matter), which surround the internal grey matter which is made of cell bodies of interneurons and motorneurons as well as unmyelinated axons and neuroglia cells (Dumont et al., 2001a; Dumont et al., 2001b; Freeman and Wright, 1953; Günther and Necker, 1995; Solomon, 1990; Weir et al., 2002). The grey matter is segmented into nine laminae; with individual specified functions (Günther and Necker, 1995; Solomon, 1990). At each individual vertebra a pair of spinal nerves, which transmit information from the rest of the body to the spinal cord and brain and vice versa, protrude from the vertebral column (Günther and Necker, 1995; Solomon, 1990).

The spinal cord is the primary neural pathway between an animal's body and its brain; as a result it is highly protected. Its first line of defense is its secure location within the vertebral column, which surrounds it in a hard calcified 'shell' of vertebra, which is also re-enforced and padded with external layers of muscle and connective tissue, creating a strong, but flexible protective cover (Günther and Necker, 1995; McLeod et al., 1964; Solomon, 1990). Within the vertebral foramina the spinal cord is encased and protected by three tissue layers (spinal meninges) and the spaces between them (Günther and Necker, 1995; McLeod et al., 1964; Solomon, 1990; Whittow, 2000). In the innermost protective space (the subarachnoid space), cerebrospinal fluid is located, as well as the arteries which supply the spinal cord with oxygenated blood (the anterior spinal artery and the paired posterior spinal arteries) (Aslan et al., 2006; Bilello et al., 2003; McLeod et al., 1964; Pryor and Shi, 2006; Solomon, 1990; Whittow, 2000). The spinal cord as well as the brain requires a high volume of blood circulation due to their high metabolic rates (Aslan et al., 2006; Bilello et al., 2003; Dumont et al., 2001a).

1.3.1.1 Injury to the avian neck

Injuries to the spinal cord are often a result of mechanical damage to the vertebral column. Due to the delicate composition of the spinal cord it is susceptible to many forms of trauma; the four main characteristic forms are compression, laceration, stretch and impact with momentary compression (Dumont et al., 2001a; Shi and Whitebone, 2006; Taylor, 1951; Weir et al., 2002). Research suggests that the grey matter appears to be more susceptible to damage than the white matter due to its consistency and increased vascularity (Dumont et al., 2001a; Dumont et al., 2001b; Walman, 1965). All of these injuries can result in complete or incomplete functional impairment, which could also lead to sensory impairment (Dumont et al., 2001a; Dumont et al., 2001b; Shi and Pryor, 2002; Taylor, 1951; Weir et al., 2002). When a trauma occurs to the spinal cord, three injury phases have been identified (Dumont et al., 2001a; Dumont et al., 2001b). The primary injury is the initial mechanical trauma to the spinal cord (e.g. burst fractures of a vertebra with bone fragments entering the vertebral foramina) (Dumont et al., 2001a; Holdsworth, 1962). The secondary injury is the mechanism of trauma which occurs as a result of primary injury, for example, neurogenic shock, hemorrhaging, and apoptosis (Dumont et al., 2001a; Dumont et al., 2001b). The final phase is the chronic neuropathology, which results from both the primary and secondary trauma (e.g. glial scarring, demyelinated axons etc.), which can permanently or semi-permanently reduce spinal cord function (Dumont et al., 2001a; Dumont et al., 2001b; Shi and Pryor, 2002). The extent of chronic neuropatholgical damage can result in major organ and body system dysfunction (e.g. paralysis, hypotension and bradycardia), which can result in the death of the animal (Bilello et al., 2003; Blight and DeCrescito, 1986; Dimar et al., 1999; Dumont et al., 2001a; Dumont et al., 2001b; Freeman and Wright, 1953; Waters et al., 1991).

The common carotid arteries supply the head and neck with oxygenated blood; however the arrangement of the carotid arteries varies significantly between avian species (McLeod et al., 1964; Perry et al., 2012; Whittow, 2000). Chickens have paired carotid arteries, which at roughly the 13th cervical vertebra meet and then together run the length of the neck (ventrally), one on top of the other, within a canal constructed between the cervical vertebrae and the longus colli muscle series (Aslan et al., 2006; McLeod et al., 1964; Perry et al., 2012; Whittow, 2000). Nearer the top of the neck (at approximately C4 or C5) the carotid arteries leave their constructed canal and separate, continuing to run towards the head, but now intertwined with the ventral straight muscles of the head and no longer in close proximity to the cervical vertebrae (McLeod et al., 1964; Perry et al., 2012). At the base of the skull, each carotid artery divides into three separate arteries; the occipital artery, the internal carotid artery and the external carotid artery (Figure 1.4) (McLeod et al., 1964; Perry et al., 2012). The occipital artery travels dorsally to the atlas and axis (C1 and C2) and supplies blood to this area and its immediate surroundings (Aslan et al., 2006; McLeod et al., 1964; Perry et al., 2012). The external carotid artery remains initially external to the skull and supplies the majority of the heads structures (except for the brain) with blood. The internal carotid artery runs through the jugular foramina to enter the brain cavity in order to supply the brain and pituitary gland (Aslan et al., 2006; McLeod et al., 1964; Perry et al., 2012).

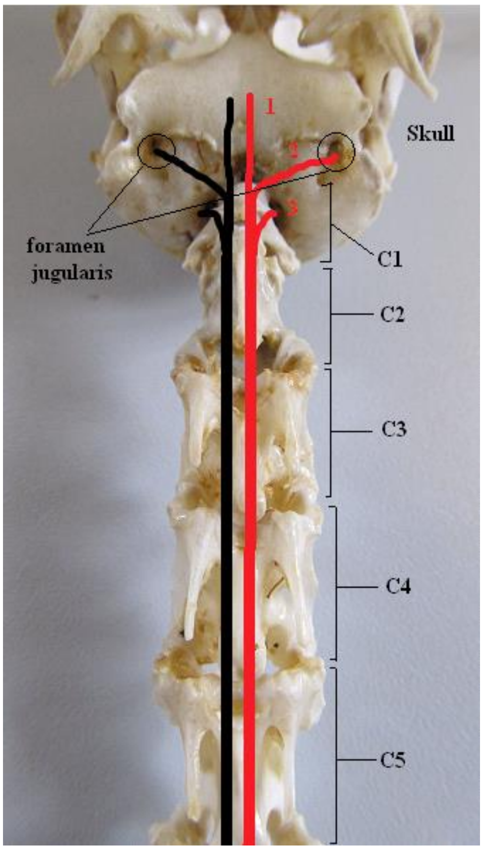


Figure 1.4 Photograph prepared and taken by the author to demonstrate the ventral surface of the cervical vertebrae and skull, demonstrating the location of the carotid arteries and their divergence into the external carotid arteries (1); the internal carotid arteries (2); and the occipital arteries (3).

Mechanical injuries to the arteries can occur as a result different types of trauma (e.g. blunt force, crushing, stretching and laceration etc.) (Abad et al., 2009; LeBlang and Nunez, 2000; Perry et al., 2012; Whittow, 2000). The injury to an artery can vary from minor to severe and in the case of carotid arteries, injuries can result in life threatening consequences (e.g. severe hemorrhaging and ischemia of the brain). Direct trauma cannot occur to the hollow space within the artery, but to the arterial walls surrounding it, which can then change the internal pressure and size of the hollow space. Arterial walls are made up of three layers: (1) the *tunica externa*, the protective outer layer (connective tissue), (2) the middle layer (*tunica media*), which is made up of both smooth muscle and elastic tissue components to provide strength and flexibility, and (3) the inner layer (*tunica intima*), which lines the internal space for blood flow and is made up of endothelial cells (McLeod et al., 1964; Solomon, 1990; Whittow, 2000). The main types of injuries to carotid arteries are listed and described in Table 1.5.

Table 1.5 A summary of the main types of injuries to arteries (Abad et al., 2009; LeBlang and Nunez, 2000; Perry et al., 2012; Solomon, 1990; Whittow, 2000).

Types of injury	Mechanical trauma causes	Description
Contusion	Blunt force impact or crushing	Bruising (minor hematoma) of the arterial wall.
Subcutaneous rupture – incomplete	Blunt force impact, crushing or stretching	The inner and middle layers of the arterial wall are torn, while the outer layer remains intact. This results in a reduction blood flow.
Subcutaneous rupture - complete	Blunt force impact, crushing or stretching	All three layers of the arterial wall are torn, resulting in blood seeping out of the vessel and into neighboring tissues and a reduction of blood flow within the vessel.
Laceration (open-wound)	Blunt force impact, resulting in crushing, twisting and tearing of the vessel	The artery is damaged as part of an open wound. All three layers of the arterial wall are broken (or the whole artery is severed) in an irregular fashion, resulting in hemorrhaging and a reduction of blood flow within the vessel.
Puncture	An object penetrates the artery	This injury can result in all types of damage reported for subcutaneous rupture or laceration.
Incision	A clean and regular shaped cut to the arterial wall (e.g. knife cut)	All three layers of the arterial wall are cut: - longitudinal cut: minimal blood loss from vessel into surrounding tissues due to the wound having a lower risk of gaping. - transverse cut: arterial wall contracts resulting in the wound gaping and increased blood loss from vessel into surrounding tissues.

Injury to the carotid arteries can have serious consequences which are highly dependent on the type of injury. The most damaging post-injury pathological effects are occlusions, pseudoaneurysms, aneurysms, and dissections (LeBlang and Nunez, 2000; Perry et al., 2012; Solomon, 1990). Each has an impact on blood flow and blood pressure within the artery (LeBlang and Nunez, 2000; Perry et al., 2012; Solomon, 1990). As the carotid arteries supply the head and neck with oxygenated blood, the effects of reduced blood flow can be life threatening because of cerebral ischemia, which can occur very rapidly due to the high metabolic rate of the brain and spinal cord.

1.3.2 The avian skull and brain

The avian skull, like the rest of the avian skeleton, is a lightweight and strong structure. Some sections of it (e.g. the occipital bone) are pneumatised (hollow with internal crisscross trusses), to provide strength with reduced weight (Hogg, 1982; McLeod et al., 1964). The main anatomical difference compared to mammalian skulls is that the avian skull lacks a 'true jaw' and in its place is the beak, which is lightweight and associated with the development of a specialised digestive system (Hogg, 1982; McLeod et al., 1964; Solomon, 1990; Whittow, 2000). Overall, the avian skull is cone-shaped (flattened on the ventral side), with the beak creating the point of the 'cone' (Figure 1.5) (McLeod et al., 1964). The acuteness of the cone-shape angle is species specific (McLeod et al., 1964), for example, in poultry the beak is fairly pointed and angular, while in ducks or geese, the angle is less acute and the beak has a more broad and blunted shape. Avian skulls also possess particularly large orbital fossa which house their relatively large eyes (e.g. ethmoid bone) (Hogg, 1982; McLeod et al., 1964; Whittow, 2000).

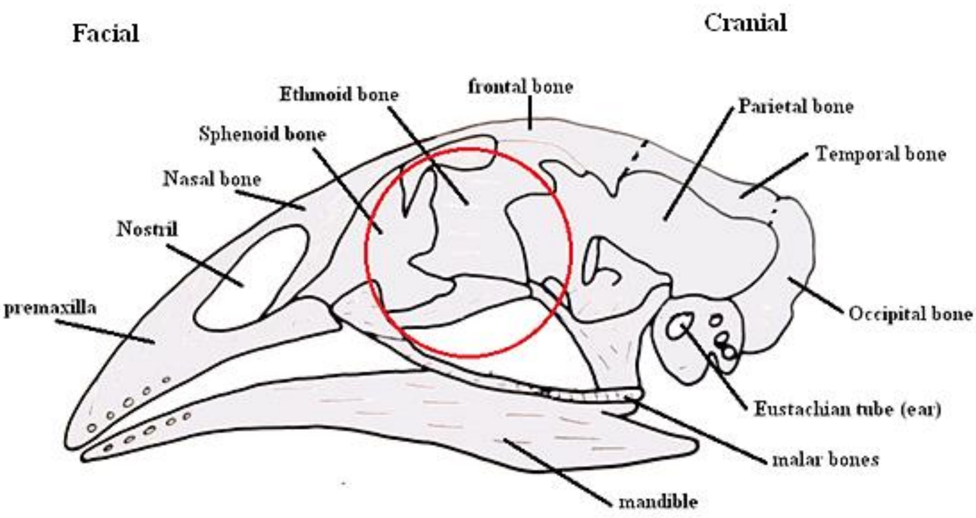


Figure 1.5 Diagram of a chicken skull with the cranial and facial bones labelled. The red circle indicates the approximate location and size of the avian eye in relation to the skull (McLeod et al., 1964).

The avian skull can be divided into two general areas, cranial and facial, both of which encompass a collection of bones which are not fused at hatching. The facial bones incorporate the bones at the front of the skull and beak and are relatively small (e.g. the nasal bone) (McLeod et al., 1964). The cranial bones form the back of the skull and form the cranial cavity (housing the brain). The main cranial bones are the occipital bone (or occipital complex), the parietal bones, the temporal bones and the frontal bones (McLeod et al., 1964). Within the occipital bone is the foramen magnum, which is the large opening at the back of the skull that the spinal cord/brain stem passes through to connect with the brain (McLeod et al., 1964). The majority of these are pneumatized, to create greater strength and protection (Hogg, 1982; McLeod et al., 1964). Previously it was believed that the cranial bones fused shortly after hatching, but more recent research has shown that the suture lines are still visible between 4-5 months of age, similar to the facial bones (Hogg, 1982; McLeod et al., 1964).

The cranial cavity houses the avian brain. Like all vertebrate brains the avian brain is a delicate structure which is made up of soft tissue (including neurons and nerve cells) and as a result it is encased in protective connective tissue layers (meninges e.g. dura mater) (Kendrick, 2007; Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). Like the meninges of the spinal cord, the cranial meninges are the location of the blood vessels and the protective cerebrospinal fluid (Aslan et al., 2006; Solomon, 1990; Whittow, 2000). The avian brain is functionally similar to the mammalian brain, being the centre of neural processing for the nervous system. It can be divided into three main regions; the forebrain, midbrain and hindbrain (McLeod et al., 1964; Solomon, 1990; Whittow, 2000). Figure 1.6 shows a cranial-caudal cross-section of a chicken head to demonstrate the position and structure of the brain in-situ.

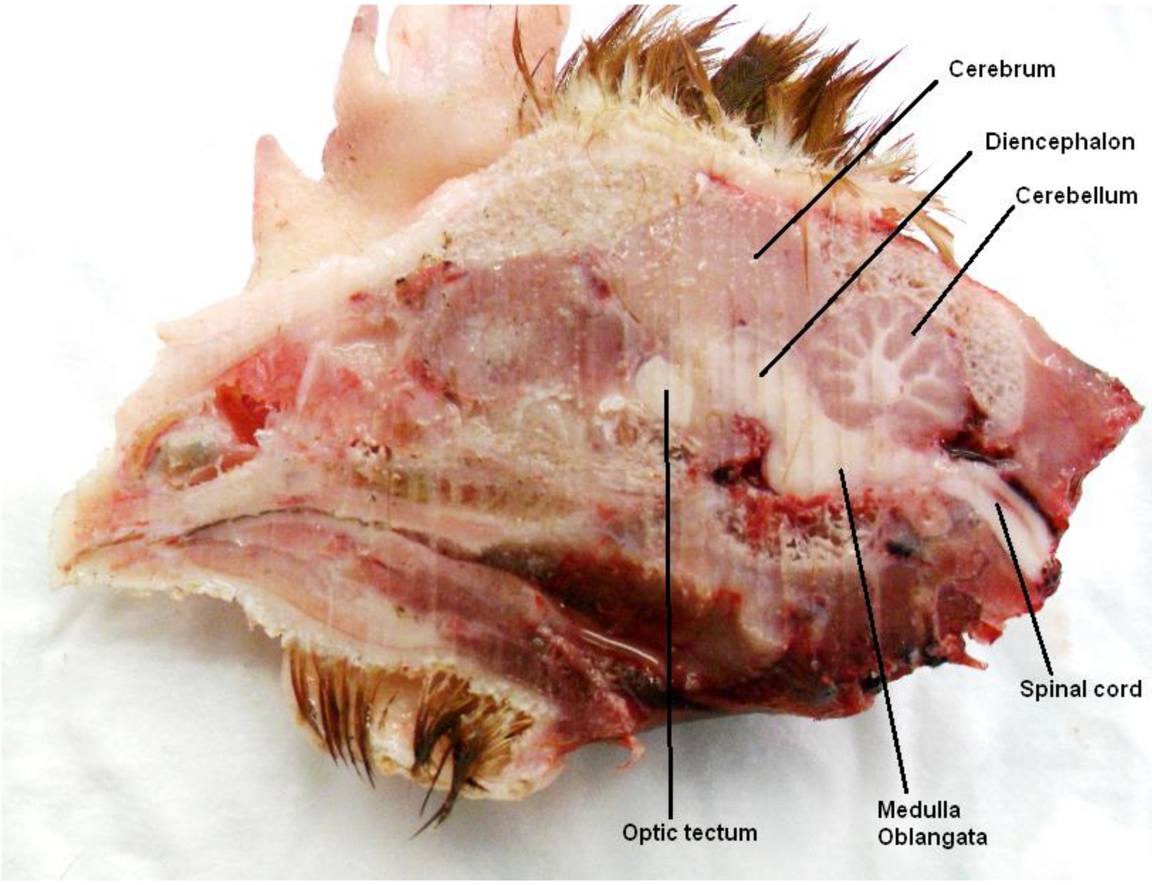


Figure 1.6 A cranial-caudal sagittal section of a chicken's head to demonstrate the location of the brain and its main identifiable regions.

The forebrain (or telencephalon) encompasses the left and right hemispheres of the cerebrum and the thalamus and hypothalamus (diencephalon), although some neuroscientists consider the diencephalon to be part of the midbrain (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). The main functions of the cerebral hemispheres are conscious motor systems, sensory processing (e.g. visual, olfactory) and cognition (e.g. memory, communication) (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). The cerebrum is the largest area of the brain in birds and mammals and it can be subdivided into four lobes (frontal lobe, parietal lobe, occipital lobe and the temporal lobe) which are specific to certain functions (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). The diencephalon has a primary function related to regulating the autonomic nervous system and endocrine glands (Solomon, 1990; Whittow, 2000).

The midbrain includes the tectum and the tegmentum (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). This is an area of marked difference compared to mammalian brains, as in birds the optic tectum is greatly enlarged and highly developed (Whittow, 2000). The primary function of the midbrain is motor orientation (e.g. moving the body towards stimuli) (Solomon, 1990; Whittow, 2000). The midbrain also possesses two important channels which connect each side of the brain (Solomon, 1990; Whittow, 2000).

The hindbrain contains several structures; the cerebellum, medulla oblongata, and the pons (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). Like the optic tectum, the cerebellum is well developed in birds. It receives wide-ranging sensory input and relays this information for its main function of co-coordinating the body's motor systems (Solomon, 1990; Whittow, 2000). The medulla oblongata connects the spinal cord to the rest of the main brain regions and is considered to be part of the structure known as the brain stem, which also encompasses the pons and the midbrain (Solomon, 1990; Whittow, 2000). The medulla oblongata has several functions: proprioception, maintenance of sleep/awake states, control of motor systems related to the nervous system, visceral organ control and regulation of 'life' mechanisms (e.g. respiration, heart rate, blood pressure etc.) (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000). The pons is referred to as the nuclei bridge between the forebrain and the cerebellum and its major functions are related to respiration, sleep states, facial movements and reflexes as well as posture (Rattenborg et al., 2009; Solomon, 1990; Whittow, 2000).

1.3.2.1 Injury to the avian skull and brain

Mechanical traumas to the head and brain of humans cause complex and multi-phased injuries (similar to that of spinal cord injuries), which can be both acute and chronic (Claassen et al., 2002; Kushner, 1998; Slivka et al., 1995). Head injuries can be classified into two groups: closed (dura mater around the brain remains intact) or open (skull and dura mater are penetrated) (Claassen et al., 2002; White and Krause, 1993; Whittow, 2000). Injuries to the brain (traumatic brain injury – TBI) can be described as diffuse or focal (Alexander, 1995; Kushner, 1998; White and Krause, 1993). Several forms of injury can result from a mechanical trauma to the head, the majority of which are summarised in Table 1.6. Head trauma which results in TBI is listed as one of the main causes of death and disability in human medicine (Kushner, 1998). As the brain is divided into regions which are related to function, it is logical to expect that focal injuries to particular regions will result in disruption to their specific functions (e.g. damage to the cerebellum results in impaired motor function). Therefore the area in which the focalised trauma occurs is very important in determining the severity of secondary damage, for example, destruction of the brain stem would result in cessation of respiration, blood circulation, etc. all of which without major medical intervention would result in death (Alexander, 1995; Dunham et al., 2012; Kushner, 1998; Oppenheimer, 1968; Walman, 1965; Widjicks, 1995).

Table 1.6 Descriptions of the most common injuries of the head and brain in humans, as a result of a mechanical head trauma (Anderson et al., 2006; Claassen et al., 2002; Kushner, 1998; Slivka et al., 1995; Solomon, 1990; White and Krause, 1993; Whittow, 2000; Widjicks, 1995; Widjicks, 2002).

Type of injury	Description
Laceration	Primarily injuries to skin layers of the scalp, which result in external hemorrhaging.
Skull fracture	The bones of the skull are cracked in either a linear or depressed manner. In some cases bone fragments can pierce the dura mater or brain tissue.
Subcutaneous hematoma	Hemorrhaging between the skin and skull.
Extradural intracranial hematoma	Hemorrhaging between the skull and the dura mater. If the hemorrhaging is as a result of a tear of an artery (a high pressure circulatory vessel) then this can result in a rapid increase intracranial pressure, which can have fatal consequences.
Subdural intracranial hematoma	Hemorrhaging of the meninges layers and pooling of blood into the spaces between them. Like extradural hematomas this type of injury can result in an increase of intracranial pressure, which could lead to fatal consequences.
Cerebral hematoma/contusion	Bruising within the brain tissue (i.e. hemorrhaging of blood vessels within the brain tissue). Like other intracranial hemorrhages there is a risk of increased intracranial pressure, and there is also a risk of cerebral oedema.
Axonal damage	Injury to axons within the brain tissue, which can range from mild to severe, dependent on the number of axons damaged as a result of the trauma. Damage to the axons results from distortions and strains which interfere with axon function and can be either semi-permanent or permanent.
Concussion	Broad term used to describe mild traumatic brain injury. Research is still inconclusive on whether concussion involves physical injury to brain or only functional impairment. Recent studies have suggested that it is a temporary impairment of neuron function within the brain through biochemical changes within cell membranes or synapses.
Contrecoup effect	The force of the mechanical trauma has caused the brain to shift or ricochet in the cranial cavity, resulting in additional injuries to the brain through impacts with the interior walls of the skull.
Cerebral oedema	The pooling of extracellular fluid within the cranial cavity, which commonly results in the brain being inflicted to an increase in intracranial pressure.

As with spinal cord injuries, the primary injury involves the damage caused as a result of the initial trauma, while the secondary injury involves the damage caused as a result of the initial trauma (e.g. intracranial hematomas, inflammation and biochemical disruption) (Alexander, 1995; Dumont et al., 2001a; Dumont et al., 2001b; Kushner, 1998; White and Krause, 1993). It is the secondary injuries which are more likely to have fatal consequences. Increases in intracranial pressure can have devastating effects and can cause herniation of the brain and if it reaches severe levels, can result in brain death (Alexander, 1995; Anil et al., 2002a; Claassen et al., 2002; Kushner, 1998). Any damage which results in disruption of blood circulation to the brain can result in cerebral ischemia, hypoxia and oedema (Claassen et al., 2002; Kushner, 1998; Slivka et al., 1995; Weir et al., 2002; White and Krause, 1993). In relation to killing methods designed to cause massive diffuse brain traumas (e.g. blunt force trauma), the resulting injuries would lead to many brain regions being irreversibly damaged with changes in intracranial pressure and blood circulation, probably resulting in death (Claassen et al., 2002; Kushner, 1998; Slivka et al., 1995; Weir et al., 2002; White and Krause, 1993). Killing methods designed to cause focal brain damage tend to target the brain stem, as this is most likely to cause fatal functional impairment (e.g. puntilla) (Dembo, 1894; HMSO, 1995; HSA, 2004; Morzel et al., 2002; Widjicks, 1995). However they are also likely to cause *contrecoup* effects and sudden changes in intracranial pressure, which can also be fatal (Kushner, 1998; White and Krause, 1993).

1.4 Small-scale on-farm killing of poultry

The development of emergency killing methods for small numbers of poultry have mainly focused on their practically, cost and availability for rapid deployment, because the majority of emergency killing needs to be done immediately, on farm sites. Common emergency killing methods for livestock are cervical dislocation (poultry), free bullet (cattle, pigs and sheep) and captive bolt (poultry, pigs, cattle, sheep), however this must be following by bleeding or pithing (only used if animal is not for human consumption) (European Council, 2009; HMSO, 1995). Livestock can also be killed by the use of veterinary drugs (e.g. overdose of barbiturate), however this is not common as only a trained individual with access to appropriate drugs (e.g. a vet) can administer this technique (European Council, 2009; HMSO, 1995). There are other techniques for emergency killing of poultry for disease control, designed primarily to cope with the much larger number of individuals involved, for example, whole-house or containerised gas killing (Gerritzen et al., 2004; McKeegan et al., 2011; Raj et al., 2006b; Raj and Gregory, 1993).

An important factor affecting the welfare of animals during emergency killing is handling prior to death. The majority of on-farm emergency killing methods require the livestock to be handled prior to the killing, and may even involve live transportation; the exception being whole house gassing of poultry. For example, target individuals may need to be separated from the rest of the livestock group and once separated they may need to be restrained. This procedure can be very stressful to the animal and should be included in a welfare assessment of the overall killing experience. Research has shown that meat quality of livestock can be affected by stress experiences of the animal prior to and at killing (Fletcher, 2002; Lambooij et al., 1999b; Lambooij et al., 1999c). Several studies have shown that improper handling and restraining of poultry can lead to physical injury (e.g. leg and wing fractures/dislocation), as well as psychological suffering through stress (e.g. prolonged handling).

As stated previously, all current on-farm killing techniques for small numbers of poultry and game birds either involve: (1) the dislocation/severing of the neck; or (2) the destruction of the brain. This allows killing method types to be logically split into two groups, as a result of the location of the trauma site. The increase in scrutiny of on-farm killing methods particularly in terms of their welfare impact has led to the design of methods which could alleviate some of the welfare concerns (e.g. extended time to loss of consciousness, operator fatigue). As the majority of concerns are based around the human operator and consistency, several mechanical devices have been designed in order to reduce variability introduced by the operator’s involvement. The devices discussed in this chapter will not include electrical stunning devices as these are not commonly used for chickens on-farm and if used have to be followed up by a killing method (e.g. MCD or exsanguination).

1.4.1 Cervical dislocation methods

The traditional method for killing poultry on-farm is MCD (i.e. necking by hand) (Sparrey et al., 2014). This method is thought to kill birds primarily by cerebral ischemia and achieves this in two stages (Bader et al., 2014; Erasmus et al., 2010a; Gregory and Wotton, 1990). Firstly, the bird’s neck is stretched in order to damage and tear the neck muscles, ligaments, connective tissue and blood vessels, as well as causing separation of the cervical vertebrae. Secondly, the twisting of the neck by tipping the birds head back causes dislocation and the severing of spinal cord (HSA, 2004; Sparrey et al., 2014), preferably between the occipital condyle on the base of the skull and the atlas (C0-C1), as this is most likely to cause maximum damage to the brain stem and potentially renders the bird unconscious immediately (Bader et al., 2014; Gennarelli, 1986; Ommaya and Gennarelli, 1974; UFAW, 2010). Some studies have suggested that there could be a concussive effect resulting from damage to the spinal cord/brain stem during cervical dislocation (Bader et al., 2014; Dumont et al., 2001a; Freeman and Wright, 1953; Harrop et al., 2001; Shaw, 2002).

Typically, MCD is performed by the operator holding the bird upside down by the legs, in one hand, with its body resting against the operator's thigh, while holding the birds head in the other hand (Figure 1.7). The HSA recommends that the bird's head is held between the index and middle finger, with the thumb and remaining fingers placed underneath the bird's chin (HSA, 2004). The other common hand grip position is to hold the bird's head between the thumb and index finger (head held against the operator's palm); secured by the bird's chin resting against the operator's thumb. From this position the bird's neck can be stretched and twisted rapidly by pulling upwards on the legs of the bird and pulling downwards using the head for grip, while also tilting the birds head upwards and back towards its tail. However, this technique may have a few variations depending on the operators experience and confidence. The HSA recommends that MCD should not be performed on birds weighing over 3 kg and it should only be performed by experienced operators, and is stipulated in the European legislation as of 2013 (European Council, 2009; HSA, 2004). Once the technique has been applied, confirmation of death is required (e.g. no rhythmic breathing, lack of evoked nictitating membrane movement), and if there is any doubt, the technique should be immediately reapplied (AVMA, 2007; HSA, 2004). As with most killing techniques, the bird should display clonic convulsions, such as leg paddling and wing flapping post-application (Erasmus et al., 2010a; Erasmus et al., 2010c; Knudsen, 2005), and this may initially hamper the confirmation of death.

Figure 1.7 Photograph of the manual cervical dislocation (MCD) method on a layer hen cadaver (64 weeks old), demonstrated by the author.

MCD is also used to kill small numbers of chicks on-farm, however the technique is a variation of the one described for larger birds (Jaksch, 1981). The ‘thumb-edge’ technique follows the same principles as described above, whereby the chick’s neck is pushed by the operator’s thumb onto the edge of a surface (e.g. table) in order to dislocate the neck. The chick dies from cerebral asphyxia, and the cervical vertebrae being dislocated, however it is more practical for application to small birds compared to MCD. Despite no research being directed at this particular technique, there is concern that it may cause crushing injuries to the neck, as well as not severing the carotid arteries as a result of minimal stretching involved in the technique, similar to concerns raised with mechanical dislocation devices which have been found to cause crushing injuries (Close et al., 2007; Gregory and Wotton, 1990).

All other cervical dislocation/severing methods for killing poultry are considered “mechanical”, as they require a tool to perform the method (e.g. blade/guillotine for decapitation or pliers for dislocation). Historically, a common killing method for poultry was decapitation, which is still used in some third world countries, and in the past it was a common dispatching method for laboratory rodents (Carbone, 1997; Cartner et al., 2007; Holson, 1992; Van Rijn et al., 2011). Decapitation is a mechanical method which involves the neck of the bird being severed as close to the head as possible, using a sharp blade in one action (e.g. with a guillotine) (Mason et al., 2009; Van Rijn et al., 2011). This should result in the bird dying from cerebral ischemia as the head is completely severed from the body preventing blood flow (Holson, 1992; Van Rijn et al., 2011). Decapitation is still a legal method for killing poultry in an emergency situation in the United Kingdom (HMSO, 1995), but it is not recommended as there are other methods which are currently perceived to be more humane (European Council, 2009; HSA, 2004). Research has shown that EEG activity, suggesting possible consciousness, persists in decapitated rats (Mikeska and Klemm, 1975).

Mechanical cervical dislocation devices are designed to achieve the same results as MCD (severing the spinal cord and carotid arteries), through the use of a mechanical aid. In most cases these devices are used for larger birds where MCD is not practical due to bird size (and/or weight) and operator ability (HSA, 2004). Table 1.7 lists the most common mechanical devices available for dispatching poultry in the UK. It is important to note, however, that many of these devices have been designed and marketed without rigorous testing of their welfare impact. Some devices which been tested have been deemed to be inhumane due to prolonged EEG activity or visual evoked responses after application, indicating that birds may be conscious post-application (Erasmus et al., 2010a; Gregory and Wotton, 1990). As a result, the new EU Regulations (1099/2009) disallow the use of any device which does not cause cervical dislocation by stretching and twisting of the neck (European Council, 2009).

Table 1.7 Descriptions of the multiple mechanical dislocating killing methods for despatching poultry on-farm, as well as the attributed advantages and disadvantages of each method.

Device	Description	Desired physiological damage	Advantages	Disadvantages
Heavy stick	The operator holds the bird upside down by both legs. A heavy stick is placed over the back of the bird's neck, as near to the back of the head as possible. The operator's feet secure the stick in this place, without applying too much pressure. When ready, the operator pulls upwards on the bird's legs while using the stick to anchor the birds head to the ground.	• Stretching and dislocation of neck. • Spinal cord, carotid arteries and surrounding tissues should all be torn. • The head should remain attached to the body post application.	• Transportable • Useable on larger/heavier birds • Minimal equipment required • Minimal biosecurity issues (no external loss of bodily fluids • Inexpensive	• Relies on operator's strength and training. • Risk of choking bird • C0-C1 dislocation not always achieved • Bird's wings not secure – risk of wing damage or injury to operator
Killing cone	The bird is placed within a poultry restraining cone mounted on a tripod (cone size dependent on bird type). Beneath the cone where the bird's head and neck protrudes, there is a hinged neck clamp. The bird's neck is placed within the clamp and when the operator is ready the clamp is pulled downwards.	See damage for Heavy stick method.	• Useable on larger/heavier birds • Moderate equipment required • Minimal maintenance of equipment required • Birds restrained in cone, not by operator • Minimal biosecurity issues (no external loss of bodily fluids	• Relies on operator's strength and training. • Risk of choking bird • C0-C1 dislocation not always achieved • Birds must be transported to device location Difficult to adjust – cone size is bird specific • Expensive (~£300)
Poultry Wringer™	The device is mounted to the floor or wall. The bird's neck is positioned within two metal V-shaped prongs, resting at the base of the 'V'. When the operator is ready the bird is then pulled by the legs away from the device, using the 'V' shape to anchor the bird's head.	See damage for Heavy stick method.	• Useable on larger/heavier birds • Minimal equipment required • Minimal biosecurity issues (no external loss of bodily fluids • Inexpensive (~£20)	• Relies on operator's strength and training. • C0-C1 dislocation not always achieved • Birds must be transported to device location

Device	Description	Desired physiological damage	Advantages	Disadvantages
Semark pliers / Humane Bird Dispatcher	This handheld device allows an operator to hold the bird in one arm and hold the device in the other. The bird’s neck is inserted in between the two blunt blades of the pliers, making sure the whole of the neck is inserted and held in place by the small ‘teeth’ at the end of each blade. To apply the device the operator closes the plier blades together by squeezing the handle.	• Blades cause dislocation and/or crushing of the cervical vertebra • The spinal cord should be severed • The head should remain attached to the body post application. • There is a risk of damage to the trachea.	• Easy to use for less strong/experienced individuals • Transportable • Minimal biosecurity issues (no external loss of bodily fluids • Inexpensive (~£40) • Minimal maintenance of equipment required	• Risk of choking bird • High risk of crushing injury to the neck and causing death via asphyxiation • Blood supply to head is not always reduced • Not adjustable to different sizes of bird • Difficult to use on conscious unrestrained birds • Banned as of January 2013 (European Council, 2009) • C0-C1 dislocation difficult to achieve
Burdizzo (Designed use: lamb castration forceps)	A handheld device with a circular jaw and blunted blades. When the jaws are open they should be placed over and under the bird’s head and positioned as near to the base of the skull as possible. To apply, the operator closes the circular jaws by pushing the handles together.	See damage for Semark pliers.	• Useable on larger/heavier birds • Minimal maintenance of equipment required • Easy to use for less strong/experienced individuals • Transportable • Minimal biosecurity issues (no external loss of bodily fluids	• C0-C1 dislocation difficult to achieve • Risk of choking bird • High risk of crushing injury to the neck and causing death via asphyxiation • Blood supply to head is not always reduced • Expensive (~£250) • Banned as of January 2013 (European Council, 2009)

Device	Description	Desired physiological damage	Advantages	Disadvantages
Turkey Pliers	A large handheld device which has ‘U’ shaped blunted blades which when closed overlap one another. The bird’s neck is placed between the jaws and once ready to apply the operator closes the jaws by pushing the handles together.	See damage for Semark pliers.	• Useable on larger/heavier birds • Minimal maintenance of equipment required • Easy to use for less strong/experienced individuals • Transportable • Minimal biosecurity issues (no external loss of bodily fluids	• C0-C1 dislocation difficult to achieve • Risk of choking bird • High risk of crushing injury to the neck and causing death via asphyxiation • Blood supply to head is not always reduced • Banned as of January 2013 (European Council, 2009)

Ideally, killing methods should render the animal unconscious immediately (AVMA, 2007; European Council, 2009; European Council, 2010). Recently, questions have been raised relating to the welfare implications of manual and mechanical cervical dislocation devices and it has been suggested that animals (including poultry) may remain conscious for a period of time post-application (Bader et al., 2014; Bates, 2010; Carbone et al., 2012; Cartner et al., 2007; Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory and Wotton, 1990; Mikeska and Klemm, 1975; Tidswell et al., 1987). Cartner and colleagues (2007) measured EEG activity in mice (N = 15) post decapitation and cervical dislocation, and their results showed that EEG activity was severely decreased approximately 10-15 seconds post-application and Visual Evoked Potential (VEP) amplitude also significantly decreased approximately 5-15 seconds post-application. They concluded that both decapitation and cervical dislocation result in rapid loss of cortical function (Cartner et al., 2007). Other studies have shown that decapitation methods have resulted in 30 seconds or more of continued spontaneous activity in the EEG, inferring that the animal is not immediately unconscious (Gregory and Wotton, 1986; Mikeska and Klemm, 1975). Both these papers highlight spontaneous EEG activity continuing for over 30 seconds post application and do not continue data collection further. Neither paper states why 30 seconds is used as the end point of data collection, but it may have been on welfare grounds under experimental guidelines. More recent research has shown that following decapitation in rats it took an average of 17 seconds for the PTOT to reduce and for the EEG to become isoelectric (Van Rijn et al., 2011) and there was an immediate change in the EEG power spectrum, with significant increases in F50 and F95 and a reduction in PTOT compared to recorded baselines (Kongara et al., 2014).

There is very little EEG activity data on genuine MCD as applied commercially, as studies exploring this killing technique in poultry have used a mechanical version instead (e.g. killing cone or Burdizzo) (Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory and Wotton, 1990). The reason for this is not clear, however it seems sensible to suggest that this was the researchers attempt to control the variation in application of MCD, which is completely dependent on the operator, because a mechanical version would increase the consistency of the treatment application. However, by using an alternative method (despite the perceived similar physiological effects), the genuine effect of MCD has not been measured. The key to MCD is the combination of the stretch and twist action to dislocate the neck, as well as severing carotid arteries and spinal cord. All mechanical versions of this method do not completely mirror this manual action. Many only do one action, the stretch (e.g. killing cone, Poultry Wringer™ and heavy stick) and the others do neither the stretch or twist and instead attempt to dislocate by separating the cervical vertebrae by forcing a blunted edge between two vertebra (e.g. Semark Pliers, Burdizzo and Turkey Pliers). Trying to separate the vertebra with one of these tools causes concerns over producing crushing injury, as if the blade edges are wrongly placed on the neck, it is highly likely the edge will be applied onto a singular vertebra, rather between two, resulting in crushing injury to the bone and reduced likelihood of the spinal cord being damaged (Gregory and Wotton, 1990).

In terms of trauma, it would be logical to assume that the method which causes more damage is more likely to kill more quickly and render the animal unconsciousness in a shorter period of time. However, the method which causes more physiological damage could also arguably stimulate more nociceptors and result in a greater experience of pain (if the bird is conscious), if even for a short period of time. In the case of cervical dislocation methods, unconsciousness is likely to occur as a result of loss of blood circulation to the brain (Aslan et al., 2006; Bilello et al., 2003; Gregory and Wotton, 1990; Pryor and Shi, 2006; Weir et al., 2002) and massive depolarisation of the neurons in the spinal cord and brain stem due to its injury, which could cause temporary disruption in function (e.g. neurogenic shock) (Bilello et al., 2003; Dimar et al., 1999; Dumont et al., 2001a; Dumont et al., 2001b). As a result, any trauma which causes rapid reduction in blood flow to the brain and extensive damage to the spinal cord in the top of the neck (C0, C1 or C2), is likely to render the animal unconsciousness quickly. Therefore manual methods which employ the twist and stretch (i.e. more trauma) could be considered more likely to achieve the reduction in blood flow and extensive damage to the neck, similar to the principal behind the design of the rope knot and the hangman's fracture in human executions (Rayes et al., 2011).

Mechanical devices which only compress the neck in order to dislocate (e.g. pliers) cause more localised trauma to the neck, similar to that of decapitation and compared to the extensive trauma caused by stretching (Bates, 2010; Cartner et al., 2007; Erasmus et al., 2010a; Gregory and Wotton, 1990; Holson, 1992; Van Rijn et al., 2011). Gregory and Wotton investigated the efficacy of Semark pliers in killing poultry and concluded that the pliers did not severe the carotid arteries, the birds showed prolonged visual evoked responses (VERs) after application and in roughly a fifth of the birds the spinal cord was not completely severed (Gregory and Wotton, 1990). This would result in continual blood flow to the brain post application, albeit somewhat reduced by aneurisms in the arteries as a result of the crush, and probably increase the time it takes for the animal to die from cerebral ischemia or to become unconsciousness (Gregory and Wotton, 1990; Perry et al., 2012; Solomon, 1990; Whittow, 2000). Several studies have demonstrated spinal cord function recovery after compression injuries if the blood supply is not significantly reduced (Dimar et al., 1999; Pryor and Shi, 2006; Shi and Whitebone, 2006) and suggest that spinal cord function is more likely to return if the compression damage is caused for a shorter period of time. When poultry are killed by pliers, the device is only applied for a short period mainly due to time constraints or the operator lacking knowledge, therefore if dislocation is not complete and the spinal cord not severed, there is the possibility of some return of spinal cord function and blood supply to the brain which could lead to prolonged suffering (Dimar et al., 1999; Dumont et al., 2001a; Dumont et al., 2001b). Similar research demonstrated that laying hens (n = 26) killed by the Burdizzo (refer to Table 1.7) suffered crushing damage to their necks, and this caused both complete and incomplete ruptures of the main blood vessels (Erasmus et al., 2010a). The authors also noted that there was a high level of variation in the location of crushing damage (i.e. which vertebrae), which appeared to be related to different operators and individual birds (Erasmus et al 2010a). Their results also showed that all hens killed by the Burdizzo were observed to have maintained their nictitating membrane reflex post application, and the authors implied that this does not suggest immediate unconsciousness (Erasmus et al 2010a).

The trauma caused by MCD is well documented in terms of its physiological effects. Due to the stretch and twist action, the injury is based around irregular tears and trauma to the neck tissue (e.g. muscle, carotid arteries, spinal cord, etc.) (Bilello et al., 2003; Brieg, 1970; Dumont et al., 2001a; Freeman and Wright, 1953; Harrop et al., 2001; Waters et al., 1991). The stretching injury to muscle, major blood vessels and the spinal cord causes greater damage to a wider area compared to a single laceration (Brieg, 1970; Perry et al., 2012; Pryor and Shi, 2006). Stretching of the carotid arteries can result in complete and incomplete tears and hemorrhaging of the arterial wall layers and surrounding tissues (Aslan et al., 2006; Bilello et al., 2003; Perry et al., 2012; Sharma et al., 2005), all of which results in change in arterial pressure and related reduction in blood flow (Solomon, 1990; Whittow, 2000). These injuries are similar to those observed post-mortem in human hanging cases (Rayes et al., 2011; Salim et al., 2006; Sharma et al., 2005). The reduction in blood flow and change in arterial pressure results in cerebral ischemia and/or hypoxia, although the speed with which these occur is highly dependent on whether both carotid arteries are completely severed (Gregory and Wotton, 1986; Gregory and Wotton, 1990; HSA, 2004). Even if the carotid arteries are not completely severed, the stretching trauma results in narrowing and occlusion (Comi et al., 2009; Gregory et al., 2012; LeBlang and Nunez, 2000; Perry et al., 2012; Solomon, 1990). MCD should also cause blood supply to the top of spinal cord to be disrupted, which causes functional impairment (Dumont et al., 2001a; Dumont et al., 2001b; Pryor and Shi, 2006) and could result in neurogenic shock (Dumont et al., 2001a).

Stretching of the spinal cord when MCD and other stretch mechanical devices are applied will cause extensive biochemical and anatomical changes within the cord itself (Blight and DeCrescito, 1986; Dimar et al., 1999; Shi and Pryor, 2002; Shi and Whitebone, 2006). Axons and their myelinated sheaths are narrowed and damaged, affecting their efficiency and function (Blight and DeCrescito, 1986; Shi and Pryor, 2002; Shi and Whitebone, 2006). Shi and Whitebone (2006) demonstrated that the various stretch strain magnitudes resulted in overall potential amplitude reductions with rapid stretch strain causing the most extensive reduction in potential amplitudes (Shi and Whitebone, 2006). In the case of MCD, the rate of stretching is very rapid and the strain is considered complete (i.e. stretching the spinal cord until it snaps), therefore the reduction in potential amplitude would also be extensive, resulting in functional impairment (Dumont et al., 2001a; Harrop et al., 2001; Shi and Whitebone, 2006).

Ideally, MCD and mechanical devices are supposed to dislocate the cervical vertebrae at C0-C1, which is the very top of the spinal cord and possibly the start of the brain stem (Solomon, 1990; Whittow, 2000), with severe implications for brain stem function and with localised temporary and/or permanent biochemical changes (Brieg, 1970; Pryor and Shi, 2006; Shi and Pryor, 2002; Shi and Whitebone, 2006; Solomon, 1990; Whittow, 2000). It has been suggested that this trauma could result in a concussive effect (Freeman and Wright, 1953; Shaw, 2002), albeit temporarily. Gregory and Wotton (1990) demonstrated that 3 out of 8 birds killed by the killing cone showed evidence of concussive effects. However, in terms of a killing method the concussive effect does not need to be permanent as long as it continues until the animal dies from cerebral ischemia/hypoxia, which with severing of the carotid arteries should occur rapidly (Aslan et al., 2006; Pryor and Shi, 2006; Shi and Whitebone, 2006). However, several authors disagree with this suggestion due to EEG data showing electrical activity in the brain for a significant period of time post-application (Cartner et al., 2007; Erasmus et al., 2010a; Gregory and Wotton, 1990). Nevertheless, it can be argued that EEG activity detected post application does not imply consciousness. EEG output reflects global electrical activity within the brain, but cannot directly indicate what the animal is experiencing, which is where evaluating the nature of the output provides further insight into conscious states. A massive trauma to an area of the brain or top of the spinal cord/brain stem may not necessarily stop all electrical signaling, but the quality of the signaling could be, in theory, seriously disrupted. Therefore any EEG activity recorded post-application of MCD or mechanical dislocation devices, could be speculated as scrambled signals spontaneously generated (e.g. hyperpolarisation and amplitude reduction of compound action potentials and depolarizing afterpotentials (Pryor and Shi, 2006)), and so may not accurately reflect information on the animal's awareness state.

There are several practical issues relating to the use of MCD and mechanical dislocating devices for on-farm killing of poultry. MCD has several practical restraints, which have resulted in some criticism of its use. Although the method requires no equipment, it does require training/experience in the technique as well as a degree of strength and confidence in its application, which after prolonged repetitive performance could be subject to fatigue (HSA, 2004; Sparrey et al., 2014). This may not be as much of concern for a physically strong male stock-worker who applies the method on a daily basis, but for an inexperienced backyard poultry keeper, where the need to perform the method is less frequent, there is concern as to whether the method is being applied correctly. MCD can be applied incorrectly in several ways, for example; (1) only partial dislocation achieved, (2) carotid arteries not severed and (3) dislocation not achieved. All three of these failures would result in a prolonged death or severe disability in the animal, as spinal cord functional impairment may be minimal and the brain would still have adequate blood supply and it is logical to suggest that this would be very stressful and painful for the bird. As MCD is purely based on the operator's ability, it is easy to see how issues of fatigue (AVMA, 2007; Canadian Council on Animal Care in science, 2010; HSA, 2004; Kingsten et al., 2005) and increases in bird size and/or weight (Erasmus et al., 2010a; HSA, 2004) could jeopardise its successful application. Chickens will usually not exceed 3 kg in weight pre-slaughter (broilers and hens); therefore this may be less of concern. However, turkeys and water fowl do exceed this weight; for example, a fully grown domestic turkey stag can weigh 30-40 kg. The physical strength required to hold a large bird upside down and in a stationary position and then pull down on the neck to cause dislocation is substantial and physically not possible for most people.

Like MCD, mechanical cervical dislocation devices have several practical limitations and Table 1.7 highlights the main problems for each individual device. The primary issue with any mechanical device is that it requires equipment, which must be purchased, maintained and the staff have to be trained in its use (although this is also the case for MCD). This causes immediate cost and time constraints related to using the device. Most mechanical cervical dislocation devices are based on fairly simple designs, which should not require extensive maintenance, but they do require occasional adjustment in order to correctly fit the bird which they are to be applied to (e.g. varying sizes of the Burdizzo or killing cone). In some cases, the mechanical device is not appropriate for more than one type of bird: for example, turkey pliers are designed specifically for turkeys and should not be used on chickens, as this may result in incorrect application and device failure, although this has not been scientifically tested. The large and wide jaws of the turkey pliers could be too large to cause vertebral dislocation in smaller birds due to several vertebrae being compressed by the jaws, resulting in crushing injury to several vertebrae and perhaps no dislocation.

Some of the devices are also quite large and cumbersome (e.g. Burdizzo and turkey pliers) and could not be a easily carried around by an operator while performing the daily check of the poultry flock, unlike the Semark pliers (Sparrey et al., 2014). There is also the issue that some of the devices are not portable at all and therefore any birds which have to be killed will have to be transported to the device's location in order to apply it. This could result in prolonged handling of the birds and an increase in stress (and pain if the bird is injured) (Chambers et al., 2001; Kettlewell and Mitchell, 1994; Petracci et al., 2010; Schilling et al., 2008; Scott, 1993). There is also the issue that on-farm killing can involve the killing of healthy animals, which may be much more difficult to handle and position in order to accurately apply the device (e.g. inserting bird's neck into plier jaws) and this could also increase the likelihood of the birds becoming stressed. Another issue with mechanical cervical dislocation devices is that they all still rely heavily on the operator's physical strength and training, albeit less so than with the manual method (MCD). Therefore, as with MCD, there is a risk of human error and fatigue which can result in inconsistent applications of the devices and the possibility of reducing bird welfare, as well as health and safety concerns for the operator.

1.4.2 Brain trauma killing methods

Killing methods for poultry which target the brain are percussive devices and can be sub-divided into penetrating and non-penetrating methods (i.e. whether or not they break the skin and cause an external wound). These are all deemed mechanical as they require a tool (e.g. captive bolt gun) for their application. There is currently no established on-farm killing device for poultry which kills by penetration, however there are several in other species (Finnie et al., 2000; Finnie et al., 2002; Gregory et al., 2007; Gregory and Shaw, 2000; Limon et al., 2009; Limon et al., 2010). There is one device which was recently evaluated as a potential on-farm killing method for poultry; the Turkey Euthanasia Device (TED) (Sandercock et al., 2012). The device showed potential for killing chickens and turkeys on-farm, however concerns were raised on the effects of the excessive forces required to retract the cowl on bird welfare (Sandercock et al., 2012). Since then, the device has been modified to prevent this issue, although independent scientific testing has not occurred to the best of our knowledge.

Practical issues with using brain penetrating devices is that they cause open wounds, which allow external loss of bodily fluids (e.g. blood and brain tissue), which is not acceptable in disease control situations and may be unpractical in a commercial poultry shed (Gerritzen and Raj, 2009; Kingsten et al., 2005; Lund et al., 2000). Issues of dissemination of central nervous system tissue into surrounding tissues and blood as a result of pithing/puntilla-like killing devices have also been highlighted (Anil et al., 2002a; Anil et al., 1999), although these risks are more important in cattle than in poultry.

Mechanical non-penetrating percussive devices are designed to strike the animal’s head in order to cause trauma to the brain. Originally, percussive devices were used to stun animals and render them unconscious as a result of the injury to the brain (Anderson et al., 2006; Gennarelli, 1986; HMSO, 1995) before then applying a kill method (e.g. exsanguination). Several devices can also be termed as stun-to-kill methods, as the brain injury they induce is severe enough to cause death (Alexander, 1995; Anderson et al., 2006; Finnie, 1993; Finnie et al., 2000; Finnie et al., 2002). However, according to the law, as the devices are designed as stunning methods, they must always be followed up by a killing method (e.g. MCD) in on-farm killing situations (or exsanguination for slaughter) unless in an emergency (European Council, 2009). There are several non-penetrating percussive devices currently available to poultry keepers for on-farm killing (Table 1.8).

Table 1.8 Descriptions of the multiple mechanical non-penetrating percussive killing methods for despatching poultry on-farm, as well as the attributed advantages and disadvantages of each method.

Percussive device	Description	Desired physiological damage	Advantages	Disadvantages
CASH Poultry Killer .22 (CPK 200)	Cartridge powered handheld device designed through a Defra funded project (Accles and Shelvoke, 2010). A captive bolt with an interchangeable head (the flat head is designed for chickens) is fired onto the top of the bird's head, using a 1 grain gunpowder cartridge. The device should be held over the top of the bird's head. When the device is fired, the bird's head should be able to have free movement after impact.	The captive bolt head should cause massive diffuse brain damage to the bird, through both primary injuries such as skull fractures, skull cavitation and brain contusions etc., but it should also cause secondary injuries such as cerebral oedema, hemorrhaging and *contra-coup* damage. This should result in the bird being concussed and rendered unconsciousness and killed.	• Consistent high powered impact. • Useable on large/heavy birds • Does not rely on the strength of the operator. • Transportable. • Adaptable bolt heads for different bird types	• Often results in penetrating wound to head. • Requires a single cartridge per bird, which has cost and time restraints (e.g. reloading). • Only a stunning method, must be followed up by a kill method (European Council, 2009). • Requires the bird to be restrained. • Health and safety constraints – requires a qualified operator. • Expensive (~£450 for device + continual cartridge costs). • Device requires regular and detailed maintenance. • Device is heavy (~2kg).
Pneumatic captive bolt gun	This device is based around a modified air powered nail gun (Draper Air Tools), but the nail cartridge has been replaced with a captive bolt and barrel. The device was tested by a Defra funded project by Raj and O'Callagahn (2001). The device's barrel is held over the bird's head and the trigger pulled to release the captive bolt. The bird's head does not require free movement post impact.	See physiological damage for CASH Poultry Killer.	• Consistent high powered impact. • Useable on large/heavy birds • Does not rely on the strength of the operator. • Transportable. • Does not require 're-loading'. • Air pressure can be adjusted dependent for bird type.	• Often results in penetrating wound to head. • Only a stunning method, must be followed up by a kill method (European Council, 2009). • Requires the bird to be restrained. • Health and safety constraints – requires a qualified operator. • Device requires regular and detailed maintenance. • Testing revealed it failed to stun birds consistently (Raj and O'Callaghan, 2001).

Percussive device	Description	Desired physiological damage	Advantages	Disadvantages
Zephyr	The device was designed by the Onatrio Ministry of Agriculture and is based around a pneumatic nail gun. The captive bolt with a 25mm convex head was attached to the device. The device should be aimed perpendicular to the top of the head, between the eye and the ear. Preliminary studies by testing scientists concluded an airline pressure of 827kPA was sufficient to cause unconsciousness and death.	See physiological damage for CASH Poultry Killer.	• Consistent high powered impact. • Does not rely on the strength of the operator. • Transportable. • Does not require 're-loading'. • Useable on larger/heavier birds	• Often results in penetrating wound to head. • Only a stunning method, must be followed up by a kill method (European Council, 2009). • Requires the bird to be restrained. • Health and safety constraints – requires a qualified operator. • Device requires regular and detailed maintenance. • Testing showed that it was prudent to fire the device at each bird twice in order to ensure the bird was rendered unconscious and dead.
Blunt Force Trauma (BFT)	A heavy instrument is swung into the bird's head. Commonly used BFT instruments are heavy pipes, bats, sticks, etc.).	See physiological damage for CASH Poultry Killer.	• Transportable. • Does not require 're-loading'. • Inexpensive • Minimal maintenance of equipment required • Useable on larger/heavier birds	• Often results in penetrating wound to head. • Requires the bird to be restrained. • Inconsistent application – force and aim based on strength and skill of operator. • Testing revealed it failed to stun/kill birds consistently (Erasmus et al., 2010a). • Testing showed that it was necessary to apply BFT twice in order to ensure the bird was rendered unconscious and dead.

The trauma resulting from a percussive device (penetrating or non-penetrating) has three aspects; external injury, primary brain injury and secondary brain injury (Claassen et al., 2002; Finnie, 1993; Krause et al., 1988; Kushner, 1998; Vink et al., 1988; White and Krause, 1993). The external injury and primary brain injury are damage caused as a direct consequence of the trauma, (e.g. skull fracture) and it is logical to assume that the greater damage caused in the direct trauma are more likely to lead to more substantial secondary injuries and fatality (Erasmus et al., 2010a; Erasmus et al., 2010b; Kushner, 1998; White and Krause, 1993). There are three main secondary injuries as a result of mechanical trauma to the brain which can be fatal. The first is contusions of the brain tissue, which occur in the localised area of the mechanical trauma, but also on the opposite sides of the brain as a result of the force of the trauma forcing the brain to shift within the cranial cavity and impact with the skull (contra coup effect) (Alexander, 1995; Claassen et al., 2002; Kushner, 1998; Machado, 2004; Oppenheimer, 1968; White and Krause, 1993). Contusions result in localised ischemia of the tissues and oedema, all of which can change the intracranial pressure (Claassen et al., 2002; Kushner, 1998; White and Krause, 1993; Williams et al., 1990). Another secondary injury to occur is hemorrhaging, which can be epidural, subdural or cerebral, all of which can disrupt the blood flow to the brain and alter the intracranial pressure (Claassen et al., 2002; Kushner, 1998; White and Krause, 1993; Williams et al., 1990). More serious hemorrhaging can result in bleeding into the cerebrospinal fluid, which can quickly lead to cerebral ischemia and vasospasm, resulting in tissue death (Claassen et al., 2002; Kushner, 1998; White and Krause, 1993; Williams et al., 1990). The third secondary injury to occur is axonal damage, where the number of axons damaged correlates with the severity of the injury and disruption to brain function (Kushner, 1998; Oppenheimer, 1968; Povlishock et al., 1983; Povlishock et al., 1992; White and Krause, 1993). Axonal damage nearer the initial injury site results in more structural damage (e.g. distortions or severing) to the axons, which cause permanent loss of function and cell death (Alexander, 1995; Kushner, 1998; Ommaya and Gennarelli, 1974; Oppenheimer, 1968; Povlishock et al., 1983; Povlishock et al., 1992; White and Krause, 1993). Diffuse axonal damage is caused by the displaced forces travelling through the tissue and tends to result in less structural damage, but semi-permanent physiological damage (e.g. hyperpolarisation) (Alexander, 1995; Kushner, 1998; Ommaya and Gennarelli, 1974; Oppenheimer, 1968; Povlishock et al., 1983; Povlishock et al., 1992; White and Krause, 1993). The extent of structural and physiological damage to the axons within the brain is theorised to be highly correlated with the length of concussion and unconsciousness (Alexander, 1995; Gennarelli, 1986; Kushner, 1998;

White and Krause, 1993). Several papers have also noted the extent of axonal damage and likelihood of unconsciousness, as well as overall brain damage, is highly related to the force of the initial trauma, with loss powerful forces producing less damage (Alexander, 1995; Raj and O'Callaghan, 2001).

Tidswell and colleagues observed brain herniation from the wound in a lamb shot by a captive bolt, although their sample size only included the one lamb (Tidswell et al., 1987). If the severity of brain damage is highly correlated with the force and diameter of the bolt, and severity of the brain injury is related to unconsciousness and loss of brain function, it seems sensible to suggest that to turn stunning devices into killing devices, the force used to drive the captive bolt must be increased (Alexander, 1995). Traumatic brain injuries are closely linked to loss of consciousness, hence this is why percussive devices were designed as stunning methods to render the animal unconscious prior to a killing method. The majority of devices are aimed perpendicular to the frontal bone of the animal, which in poultry tends to be between the eyes at the level of the ear, therefore the primary force of the bolt is fired ventrally into the anterior area of the intracranial cavity, focusing the primary brain damage to the frontal lobes (forebrain) (Solomon, 1990; Whittow, 2000). As the forebrain has been demonstrated to have primary functions relating to cognitive and sensory processing, it is logical that massive damage to this area would result in immediate unconsciousness (Solomon, 1990; Whittow, 2000). However, percussive devices used on fish are aimed at the back of the head, since shots fired in this area result in a more effective stun (Morzel et al., 2002; Robb et al., 2000).

Gregory and Wotton (1990) tested a device, similar to the CASH Poultry Killer (see Table 1.8), and they demonstrated that 5/8 birds showed greatly reduced VERs post-application and their peak to peak amplitude reduced by 74%. However they did note that if the birds were ventilated post device application, some VERs returned, albeit in a simplified form, perhaps suggesting that the loss of brain function was incomplete. The CPK has no published data on its effectiveness, but it was recently evaluated on a Defra project (MH1045), which showed it to be a highly effective killing method, with immediate loss of reflexes indicating consciousness (e.g. jaw tone), however EEG recordings were not performed due to the EEG electrode interfering with the device application (DEFRA, 2014). Several studies have also concluded that the captive bolt renders birds unconscious immediately or rapidly post application and this infers that they are a humane method of killing (Erasmus et al., 2010a; Gregory et al., 2007; Gregory and Shaw, 2000; Lambooij et al., 1999b; Raj and O'Callaghan, 2001). Work carried out by Raj and O'Callaghan (2001) on poultry demonstrated the importance of bolt size, bolt power and the penetration depth in determining the effectiveness of the CASH pneumatic captive bolt gun and their results are supported by similar findings in other species (Daly, 1987; Finnie, 1993; Finnie et al., 2002; Gregory et al., 2007; Lambooij et al., 1999b). They also concluded that the minimum requirements to ensure a successful stun and kill were a minimum bolt diameter of 6mm and a penetration depth of 10mm, fired at a 90º angle to the top of the head with a pressure of 827kPa (Raj and O'Callaghan, 2001). It has also been shown that firing the bolt at any other angle other than 90º to the top of the head resulted in inconsistent and an ineffective stun (Raj and O'Callaghan, 2001).

Non-penetrating percussive devices have also been shown to have practical issues, like other mechanical methods. The first important issue is that as the device is more complex, errors in its use may be more likely. For example, as several of the devices have interchangeable bolt heads appropriate for different species or size of birds, the use of the correct bolt is the responsibility of the operator, as is firing the bolt at the correct pressure and angle to the head (Erasmus et al., 2010a; Raj and O'Callaghan, 2001). Therefore incorrect application of the device is possible due to human error, as well as the use of the device on atypical birds (e.g. runt birds can be considerably smaller than their healthy counterparts), where the operator must make informed decisions as to what is appropriate or not. However the use of these mechanical complex devices does have some advantages in terms of practicality, for example each firing results in the bolt impacting with the head at a consistent velocity and force (Morzel et al., 2002), reducing the risk of fatigue. There is also the issue that a method of restraining the animal is required in order to accurately aim and fire the captive bolt; in the case of poultry this could be through another operator manually restraining them or through the use of a cone. However, the use of the cone reduces the transportable aspect of the percussive devices, and the use of another operator increases the cost and man power required on site.

The application of the device can also result in an open wound on the top of the head, which results in external blood loss and blood splattering within the shed, which creates biosecurity issues which are difficult to manage when other birds are within close proximity (Mumford et al., 2007). Use of percussive devices can also pose health and safety concerns for the human operator, as well as consideration that these devices are deemed firearms and need to be handled by trained and qualified operators (European Council, 2009; HSA, 2004). Another primary issue with percussive devices is that as they were originally designed to stun, therefore currently by law all devices must be followed by a kill method (e.g. MCD), unless in an emergency, which makes their use inefficient in terms of time and cost. There is also the disadvantage that these devices require re-loading and/or cocking after each firing, as well the cost of cartridges, which increase the time and cost required to dispatch a group of birds. Originally, these devices were designed to render an animal unconscious for a period of time to allow a killing action to be applied (e.g. exsanguination). If one takes the attitude that a humane method of killing renders an animal unconscious immediately on application and keeps them unconscious until death has occurred, then the damage incurred to the brain must be irreversible and substantial enough to accomplish this.

1.5 Conclusion

This review has described several mechanical devices currently available to kill poultry on-farm and highlights the concerns attributed to each of them. Many devices have not been thoroughly researched in terms of their effectiveness and time to loss of consciousness for the target animal and available studies are limited by the use of indirect measures (e.g. loss of reflexes and loss of electrical activity in the brain). In particular, there is currently no published data on EEG activity post-application of genuine MCD. The lack of scientific scrutiny of several of the current mechanical devices during their manufacture (and before marketing) is also of great concern, as retrospective work has shown that some devices are ineffective and/or inhumane. With the change in European legislation on the Protection of Animals at the Time of Killing (1099/2009) (European Council, 2009), which will restrict the use of MCD and bans the use of some mechanical devices (e.g. pliers), there is a great need for new or modified mechanical devices to be designed and tested on poultry in order to provide the industry with humane on-farm killing methods.

1.6 Project aims

The overall aim of this project was to design and develop novel mechanical killing methods for poultry, which comply with the new European Council Regulations on the Protection of Animals at the Time of Killing (1099/2009) (European Council, 2009). It was essential that the new mechanical method be developed with the support of the poultry industry in order to help advertise it and increase its success if the developmental research shows that it is effective and humane. The objectives of the project were to:

(1) Identify the currently used killing methods on-farm and gauge the reasons for their use as well as the lack of use of others.

(2) Modify or design novel mechanical devices for killing poultry, which would then be tested on cadavers, anesthetised, and finally conscious birds (with modifications made in between to improve their function).

(3) Identify the most promising mechanical device from the previous stages in terms of reliability, humaneness, and consistency and then take it forward to on-farm commercial testing in comparison with MCD, with multiple operator use.

Collectively, these objectives contributed to the final aim which was to identify a successful and novel mechanical killing device for poultry which could be marketed to the poultry industry to provide them with a more humane method of killing compared to their current practices.

2 Survey of on-farm killing methods used in the United Kingdom

2.1 Introduction

The method used to despatch birds has great significance for bird welfare, as well as for operator efficiency and health and safety. Due to the large range of killing methods available to the industry and hobby poultry keepers (e.g. manual cervical dislocation, mechanical dislocation using a killing cone, blunt force trauma, Semark pliers, CASH Poultry Killer (CPK 200), etc.) an investigation of what methods are being used and why was carried out. There is also concern that several methods currently available are associated with a number of welfare concerns in relation to length of time taken to achieve loss of consciousness and death (Erasmus et al., 2010a; Erasmus et al., 2010c; Gregory and Wotton, 1990; Lambooij et al., 1999b; Raj and O'Callaghan, 2001), as well as possible practical issues affecting performance (e.g. maintenance).

Although there are recommendations for which methods should be used (European Council, 2009; HSA, 2004) there is no previous work which surveys the killing methods used for poultry on-farm. The most common method for despatching birds in the poultry industry is widely considered to be manual cervical dislocation (MCD); however there is no quantitative evidence to support this. The suggested reasons for this preference are speculative and anecdotal. There is also the question as to why MCD is the dominant method, and what barriers have prevented the wide adoption of newer methods, such as the CPK. Sparrey and colleagues (2014) produced a review which documented current and novel on-farm killing methods for poultry, but this study provided no evidence of how common their use was.

The European Council Regulations (1099/2009) (European Council, 2009) on the Protection of Animals at the Time of Killing became active in January 2013 and has heavily restricted the use of MCD in poultry. As a result, there is a great need for alternative methods. It is important to understand the industry’s response to the legislation change as well as evaluating MCD’s popularity and the reasons why currently available alternative methods are not being used. If the current alternatives are not appropriate for use (i.e. not humane, unpractical, etc.), then research must focus on what the industry would accept as an alternative. Therefore the aims of this survey study were to: 1) establish which on-farm killing methods were currently being used by the poultry industry in the UK; 2) establish the reasons for these choices; and 3) determine attitudes to the legislation change (EU 1099/2009) and preferences for alternative killing methods.

2.2 Methods and materials

2.2.1 Questionnaire design

Two short questionnaires were designed using Snap® survey software (Snap Surveys Ltd.): one for distribution to members of the British Poultry Council (BPC) and the second to the members of the British Egg Industry Council (BEIC). Electronic copies of the questionnaire were created and distributed through the BPC and BEIC member emailing lists. The questionnaires were designed to be completed anonymously by individual poultry stock-workers working in the United Kingdom. Both councils required approval of the questions included in the questionnaires prior to circulation to their members. Originally both councils were given identical questionnaires however BPC requested the removal of the last two questions regarding the EU legislation change.

Both questionnaires contained 10 questions, with the first eight being identical between the versions. The first section asked the stock-workers for general information about themselves as well as their employing company. The second section focussed on ascertaining the current killing method used on-farm (including "other" option), as well as the reasons for this choice. They were also asked to identify their personally preferred killing method and their reasons for this choice. Pliers were separated from other mechanical cervical dislocation methods due to the crushing aspect and the different mechanics of this technique, compared to other mechanical cervical dislocation methods (refer to Section 1.4.1). The final section differed between the two surveys: for the BEIC questionnaires the section asked whether the individual was aware of the EU legislation change (EU 1099/2009) and whether this would affect the killing method choice on their farm. For the BPC questionnaires the final section asked whether the stock-workers would consider using a new killing method, and to explain their answer. Both questionnaires had a free text comment box at the end allowing the participants to include any of their suggestions or ideas for a new killing method. Both questionnaires are provided in Appendices 1 and 2.

2.2.2 Questionnaire distribution

The questionnaires were distributed electronically between November 2011 and February 2012 and a deadline for returned completed surveys was set for the end of February 2012. Surveys could be returned my email or post. There were a total of 68 member organisations and associate members of BPC, which covers approximately 90% of the British poultry meat producers. BEIC was made up of 11 member organisations which produce 85% of UK eggs. All member organisations were asked to distribute the survey amongst their employee stock-workers. The total number of actual recipients is not known, due to the large variation in employment numbers within each organisation.

2.2.3 Statistical analysis

Paper and email responses were manually entered into an Excel spreadsheet and were analysed in Genstat (14th edition) and Minitab 15. The BEIC and BPC responses were combined for identical questions, but kept separate for non-identical questions. Data were maintained at individual stock-worker level and not grouped within organisations. Frequency differences for the current and preferred killing methods were analysed using Chi-Square tests in Minitab 15. The frequency differences for reasons of preferred killing method were sub-divided into killing methods and then Chi-Square tests were used to analyse the differences within each killing method. Results were statistically significant at $P \leq 0.05$, and tendencies at $P \leq 0.10$.

2.3 Results

In total, 217 questionnaires were completed from worker at 56 individual farms. Table 2.1 shows the numbers and gender of stock-workers by the primary bird type that they worked with. Approximately 67.7% of stock-workers were sourced from three major poultry producers. The majority of respondents were male (95.4%) and worked with meat-type birds (92.2%). The mean number of years of experience as a poultry stock-worker was 21.8±0.8 years; Figure 2.1 demonstrates the range of years of experience across the poultry types. Note that one broiler stock-worker had 60 years of experience.

Table 2.1 Demographic profile of survey respondents.

Bird type	Survey distributed (BPC/BEIC)*	Gender of stock-workers Male	Female	TOTAL
Broiler	BPC	131	7	138
Duck	BPC	6	0	6
Geese	BPC	1	0	1
Laying hen	BEIC	15	2	17
Turkey	BPC	54	1	55
Total	-	207	10	217

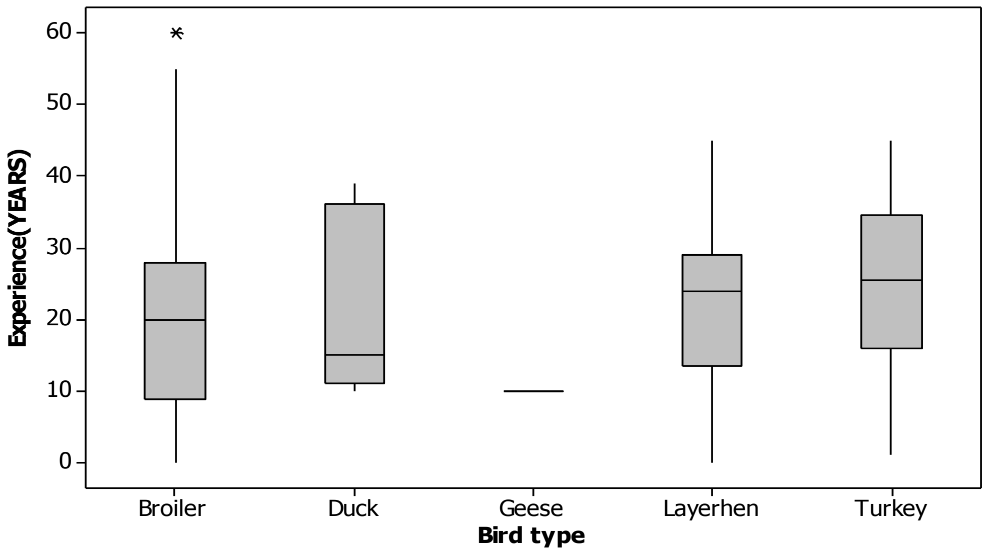


Figure 2.1 Range of years of experience of stock-workers across different bird types.

The distribution of responses of the currently used on-farm killing methods is shown in

Figure 2.2, and demonstrated that the majority used MCD for emergency on-farm killing of poultry (88.0%) (χ^2 = 631.2$_{(4,217)}$, P < 0.001). Of the same stock-workers, MCD was also the most preferred killing method (98.6%) (χ^2 = 681.4$_{(4,217)}$, P < 0.001). Table 2.2 shows the distribution of currently used on-farm killing methods across the five bird types included in the survey results. MCD is the most used kill method across all five bird types for emergency on-farm killing. For ducks, geese, and layer hens MCD was the only method used for on-farm killing by the stock-workers included in the survey. Turkey stock-workers showed the greatest variation in on-farm killing methods used, with pliers (a form of mechanical cervical dislocation) being the second most commonly used method. Interestingly, all except one female stock-worker currently used and preferred MCD as a killing method for turkeys compared to other killing methods suggested. The exception was a worker who used and preferred electrical stun to kill methods and this was the only female to work with turkeys.

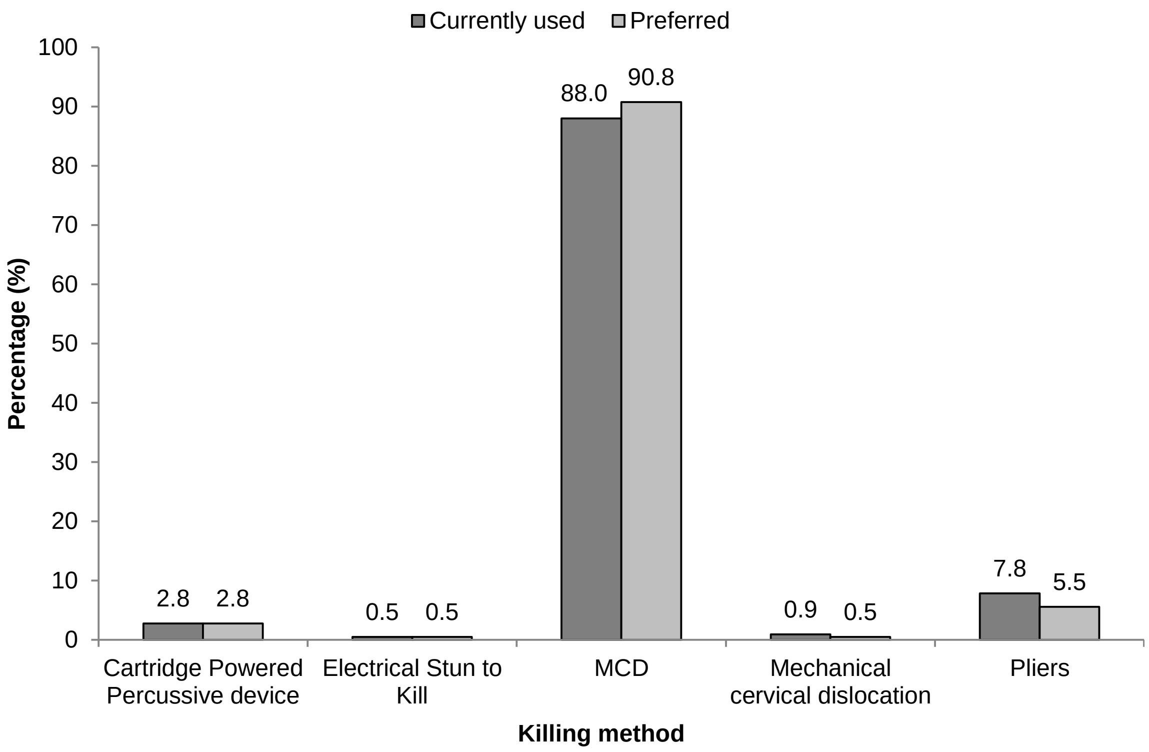


Figure 2.2 Distribution of the methods currently used on-farm for dispatching poultry, as well as the preferred killing method for stock-workers. Killing method categories which 0% of respondents used or preferred are not shown (pneumatic percussive devices, decapitation and other).

Table 2.2 Percentage distribution of stock-workers currently using various on-farm killing methods per bird type.

Bird type	Cartridge powered percussive device	Electrical stun to Kill	MCD	Mechanical cervical dislocation	Pliers	Pneumatic percussive device	Decapitation	Other
Broiler	0.0	0.0	99.3	0.7	0.0	0.0	0.0	0.0
Duck	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Geese	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Laying hens	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Turkey	10.9	1.8	54.5	1.8	30.9	0.0	0.0	0.0

Table 2.3 shows the distribution of preferred on-farm killing methods across the five bird types. There are slight differences compared to the currently used killing methods, suggesting that some stock-workers (N=16), were not using their preferred killing method. Of those sixteen, ten preferred MCD over their currently used method, which included pliers, cartridge powered percussive device and mechanical cervical dislocation; and only four preferred an alternative to MCD (i.e. CPK or pliers). The biggest difference between currently used and preferred methods was seen in respondents who currently used pliers (7/16 stock-workers).

Table 2.3 Percentage distribution of stock-workers preferred on-farm killing methods for poultry per bird type.

Bird type	Cartridge powered percussive device	Electrical stun to Kill	MCD	Mechanical cervical dislocation	Pliers	Pneumatic percussive device	Decapitation	Other	Total N
Broiler	0.7	0.0	98.6	0.0	0.7	0.0	0.0	0.0	138
Duck	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	6
Geese	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	1
Laying hens	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	17
Turkey	9.1	1.8	67.3	1.8	20.0	0.0	0.0	0.0	55

For the preferred on-farm killing methods, stock-workers were asked to identify reasons for this preference. Table 2.4 shows the reasons that certain killing methods were preferred. All killing methods, except mechanical cervical dislocation, were judged to be humane by 80-100% of respondents. The primary reasons for preference (>75%) for MCD, cartridge powered percussive devices, and electrical stun to kill were time efficiency and humaneness. The primary reasons to prefer mechanical cervical dislocation were time efficiency and low maintenance, while for the pliers, humaneness, easy to learn/use, and high success rate were the primary reasons. When asked if they could suggest any improvements to their preferred killing method 93.5% of respondents said “no” and the remaining respondents did not fill in the suggestion box.

Table 2.4 Preference reasons attributed to each preferred killing method.

Reasons for preferences (% respondents)	Cartridge-powered percussive device	Electrical Stun to Kill	MCD	Mechanical cervical dislocation	Pliers
Time efficient	67.0	100.0	72.0	100.0	50.0
Humaneness	83.0	100.0	82.0	0.0	83.0
Easy to learn/use	0.0	0.0	54.0	0.0	92.0
Cost efficient	17.0	0.0	39.0	0.0	67.0
Low maintenance	17.0	0.0	38.0	100.0	67.0
High success rate	50.0	0.0	69.0	0.0	83.0
Low fatigue risk	33.0	0.0	12.0	0.0	58.0
Health & safety risk	33.0	0.0	22.0	0.0	67.0
Other	0.0	0.0	2.0	0.0	0.0

The survey distributed by the BEIC demonstrated that only 37.5% of stock-workers (N=16) were aware of the new EU legislation (EU 1099/2009) coming into force in January 2013. The BPC-circulated survey demonstrated that only 35.3% of stock-workers would consider an alternative mechanical kill method rather than their currently used method, and with those explaining their consideration of alternatives basing their choices on the need for killing larger/heavier birds (15.5% of respondents) and large numbers of birds (7.0% of respondents).

2.4 Discussion

Although a disproportionate number of respondents were from the poultry meat industry, the results clearly demonstrate that MCD was the most commonly used and the preferred killing method for poultry, irrespective of bird type, in the UK prior to the legislation change of the EU Regulations on The Protection of Animals at the Time of Killing (European Council, 2009). The results confirm assertions in previous articles that MCD is the primary or traditional on-farm killing method for poultry (Mason et al., 2009; Sparrey et al., 2014). The preference for and use of MCD was very prevalent in all bird types (>98%), except for turkeys (>54.5%), where several other methods were also used. The reasoning for the lower preference and usage of MCD is very likely to be due to the size and weight of turkeys. Male turkeys can weigh up to 18 kg prior to slaughter, and in these large birds, MCD is not appropriate due to the operator’s strength and skill required to lift/hold the bird, as well as to apply the neck stretch correctly. Several advisory bodies do not recommend MCD for larger birds and suggest alternative methods (e.g. HSA, AVMA) (AVMA, 2007; HSA, 2004; Mason et al., 2009). Surprisingly, very few stock-workers use captive bolt methods for on-farm killing of poultry, despite extensive research demonstrating their high success rate and rapid loss of consciousness in poultry (Erasmus et al., 2010a; Finnie et al., 2000; Finnie et al., 2002; Gregory and Shaw, 2000; Raj and O'Callaghan, 2001).

Interestingly, over 80% of stock-workers considered their preferred killing method to be humane, except those who selected mechanical cervical dislocation (e.g. heavy stick), where none of the respondents chose humaneness as a reason for their preference. This suggests that they do not perceive this method as humane. By contrast, only 69% of respondents who preferred MCD specified this preference was because of a high success rate, suggesting that their perception of the method is that it does not always work or that it requires multiple attempts (which calls into question its humaneness), suggesting that perhaps the method does not always work or it was not considered an important reason if all those surveyed considered all methods to have a high success rate. Similarly, the same pattern was shown with the cartridge powered percussive device. However, the lack of selection of some reasons (e.g. high success rate) may be attributed to the respondents only selecting a few primary reasons for their preference or selecting reasons which distinguish one method from another, despite instructions stating to select all reasons. Therefore the lack of selection of a reason does not necessarily infer a negative response.

Concerns related to MCD which have been raised previously by research are consistency in application (i.e. training) and fatigue risks (Mason et al., 2009; Sparrey et al., 2014), and this was supported by the result that only 12% of stock-worker respondents stated the preference for MCD was based on low fatigue. Approximately half of the respondents who preferred MCD reported it to be easy to use/learn, which has been previously reported as a primary reason for its preferred use (Mason et al., 2009; Sparrey et al., 2014). For all other methods, which were mechanical, and therefore designed, in theory, to reduce fatigue, low fatigue was not a reason selected for preference either (e.g. CPK, mechanical cervical dislocation), suggesting that stock-workers do not perceive a mechanical aid as reducing their energy expenditure. Plier devices were considered the most easy to use (92% respondents), which may be attributed to their simple design.

Despite concerns around the reported time to unconsciousness when using plier devices and MCD (Erasmus et al., 2010a; Gregory and Wotton, 1990), the responses from the survey demonstrated that pliers were the second most popular method for despatching poultry on-farm in the UK. This preference was strongly demonstrated in that 50% of all respondents picked all the provided reason categories (e.g. time efficient, humaneness, cost efficient, low fatigue risk, etc.), suggesting this method is well liked, particularly in the turkey production work-force. However, these results highlight concerns that stock-workers may perceive certain on-farm killing methods as effective and humane, but which under research scrutiny are found not to be. This could reflect a lack of understanding and knowledge in stock-workers regarding what should occur (e.g. behaviours/reflexes) after successful application of a method. Several of the reflexes used to infer consciousness and brain death (e.g. nictitating membrane) (Erasmus et al., 2010c; Sandercock et al., 2014; Shaw, 1989) require training to be correctly identified or in some cases equipment. For example, the pupillary reflex is the constriction of the pupil in response to a light shone at the eye (Croft, 1961; Erasmus et al., 2010c; Sandercock et al., 2014), as well as relaxation and dilation once the light stimulus is removed. Resulting from successful killing method and brain death, the reflex should be completely lost and the pupil should be fixed and dilated, irrespective of light stimulus (Croft, 1961; Erasmus et al., 2010c; Sandercock et al., 2014; Shlugman et al., 2001). However, in some cases the pupil can become fixed and constricted (i.e. pin-prick pupil) (Larson and Sessler, 2012; Shlugman et al., 2001), nonetheless still defined as unresponsive, which could be mistaken for loss of the reflex and diagnosis of a successful kill. Some research has demonstrated that post-operative patients reporting acute pain, display constricted pupils irrespective of light stimulus (Aissou et al., 2012; Larson and Sessler, 2012). The incorrect diagnosis of a successful kill due to lack of knowledge of brain death indicators would have severe consequences for bird welfare on an individual and population level. It would be prudent to suggest that all stock-workers should be trained in identifying and observing the correct pattern of reflex/behaviour loss which are indicative of a successful kill, however further work needs to be done in order to refine the pattern of behaviour and reflex loss as a chicken dies.

The demographic data collected here demonstrated that the poultry industry work-force is male dominated and the average reported length of experience suggests that new individuals are rarely entering the industry. Due to uneven numbers of respondents with different levels or experience and gender, the analysis of these factors on currently used and preferred killing methods was restricted, although it could be suggested that as MCD is considered to be a common method, and as the work-force is an older generation and is not being replenished, it is less likely that novel methods will be easily adopted. This was also highlighted by the responses in the suggestion box for improvements to their preferred method, where the majority stated “no” or did not comment at all, This suggests that the respondents were either happy with the killing method they used or were not interested in modifying it. Further, 64.7% of respondents to the BPC survey stated that they would not consider using a mechanical alternative method.

Disappointingly, the sample of respondents was limited in terms of bird type, with the majority of respondents working with broilers and to a lesser extent turkeys, and limited numbers working layer hens, ducks or geese. Therefore the reported currently used and preferred killing method (i.e. MCD) for these bird types may not be a true representation of all stock-workers. This may be particularly true for people working with geese, which are a large and heavy domestic bird, where it might be logical to assume mechanical methods would be used instead of MCD and it is recommended to do so in the literature (HSA, 2004).

In conclusion, MCD was the most prevalent on-farm killing method for poultry, particularly in chickens, irrespective of type (meat or egg), in the UK, on the basis of a limited-response survey. It is not only the method of choice for individual stock-workers; it is also the most used and endorsed by individual companies in their standard operating procedures. Alternative available on-farm killing methods were rarely used, suggesting either a lack of willingness to try the methods or that following pilot use, the stock-workers did not prefer them to MCD. This survey highlights resistance from members of the British poultry industry to consider alternative killing methods, which could place limitations on future planning for adapting their on-farm killing methods in response to the European legislation change (EU 1099/2009) (European Council, 2009). Finally the strong preference for cervical dislocation methods (e.g. MCD and pliers) and the reasons for this preference should be considered when developing novel methods for on-farm killing.

3 General Methodology: design of the devices

3.1 Introduction

There are currently several on-farm killing methods available to the poultry industry in the United Kingdom (UK), however in reality only a few methods are in use. In particular, manual cervical dislocation (MCD) has been shown to be the most prevalent method in the UK. However, following the recent changes to European legislation with the Protection of Animals at the Time of Killing (European Council, 2009), the use of several of these methods has been restricted. For example, MCD can only be applied to birds which weigh under 3 kg and can only be applied on to a maximum of 70 birds per person per day, and any method which causes crushing injury (e.g. Semark pliers) is prohibited. There are also restrictions for captive bolt methods (e.g. CPK), which are defined as stunning methods only, and therefore in non-emergency cases, the “stun” method must be followed by a killing method (e.g. exsanguination or MCD). Therefore there is a need for the development of novel methods for killing poultry on-farm, which complies with the new legislation, but is also proven to be humane and practical. Information provided through the literature review (Chapter 1) and the results from the questionnaire (Chapter 2.3) provided insight into criteria which were used to develop and identify mechanical methods for killing poultry on-farm. All methods developed were designed to comply with the current European legislation on the Protection of Animals at the Time of Killing (European Council, 2009). A total of four mechanical methods for killing poultry on-farm were developed or modified, with two of the methods designed to kill by head trauma and the other two as a result of cervical dislocation. Several studies have demonstrated the high kill success rate of head trauma using captive bolt devices and reported rapid loss of consciousness in poultry, as well as other species (Erasmus et al., 2010a; Finnie et al., 2000; Gregory et al., 2007; Gregory and Shaw, 2000; Lambooij et al., 1999b; Raj and O'Callaghan, 2001). However, the questionnaire study described in Chapter 2 demonstrated the lack of uptake of current captive bolt methods (e.g. CPK), with primary issues around cost, training and biosecurity (Accles and Shelvoke, 2010; Galvin, 2005; Gerritzen and Raj, 2009; Kingsten et al., 2005; Lund et al., 2000; Sandercock et al., 2012). Therefore the aim was to modify two different captive bolt devices, with permission from their original inventors, in order to overcome some of these issues, to make them more appealing to the poultry industry. One such device was the

Rabbit Zinger™ (Pizzurro, 2009a; Pizzurro, 2009b), which was chosen primarily because the bolt is fired by stored energy in stretched rubber tubes rather than cartridges or compressed air canisters, thus making it much cheaper to use than other captive bolt methods. The second proposed device was the Armadillo®, which was originally designed to kill similar to a punctilio method (Limon et al., 2009; Limon et al., 2010; Limon et al., 2012) and was based around a simple mechanical design. The final two devices were designed and developed following the high preference for MCD and mechanical dislocating pliers. Despite some concerns in regards to these methods (e.g. inconsistency in manual application and crushing injury and time to loss of consciousness) (Bader et al., 2014; Cartner et al., 2007; DEFRA, 2000; Erasmus et al., 2010a; Gregory and Wotton, 1990), dislocating pliers were modified in an attempt to reduce the risk of crushing and a novel glove device was developed in order to mimic and standardise the action of MCD.

3.2 Pilot work

Schematic measures of the head and neck of different types of poultry were recorded for a total of 205 live birds. Five distinct measures were taken from the head and one from the neck, using digital callipers (Figure 3.1). The calculated means were then used to accurately modify and develop the devices for despatching the primary poultry types (e.g. broilers, layer hens and turkeys) at various production stages. The results are reported in Table 3.1.

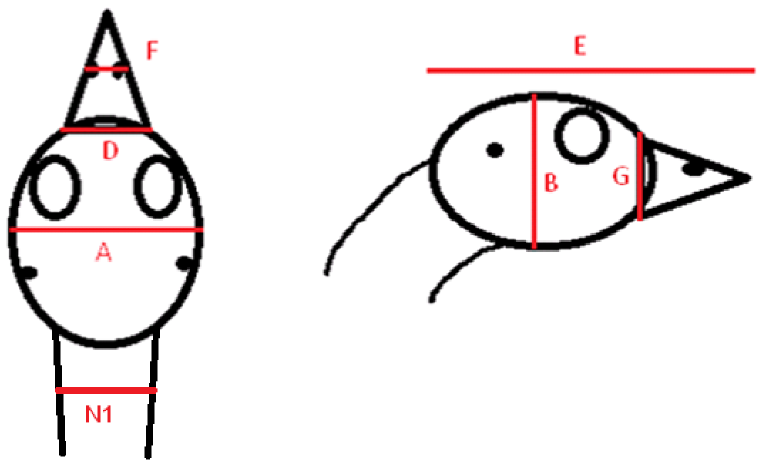


Figure 3.1 Schematic showing measures taken from live birds: A = width of head; B = lower jaw to top of skull; D = width of base of beak; E = base of skull to front of beak; F = width of beak at central nostril level; G = depth of beak; and N1 = width of neck.

Table 3.1 Mean (±SE) of multiple head and neck measures (mm) of various bird types and range of ages (days). See Figure 3.1 for explanation of areas measured.

			Area measured (mm)											
Bird type	**Age**	**N**	**A**		**B**		**D**		**E**		**F**		**N1**	
			Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Broiler	31	17	32.3	1.1	33.6	0.7	23.5	0.6	62.6	13.4	13.5	0.3	29.1	0.3
Broiler breeder	13	10	22.6	0.1	25.3	0.3	16.5	0.4	52.1	0.8	9.7	0.2	22.4	0.6
Broiler breeder	18	10	24.0	0.1	26.2	0.3	17.3	0.2	56.1	1.0	9.4	0.2	21.9	0.4
Broiler breeder	25	10	25.9	0.2	27.8	0.6	18.7	0.2	61.7	0.5	10.8	0.1	26.1	0.8
Broiler breeder	48	10	31.1	0.6	32.5	0.8	21.1	1.1	75.4	2.7	12.4	0.3	25.8	0.7
Broiler breeder	54	10	31.2	0.7	32.1	1.0	22.7	0.6	77.6	1.6	12.5	0.4	27.4	0.7
Broiler breeder	61	10	31.6	0.9	32.8	0.8	23.4	0.6	78.0	2.4	13.5	0.2	29.3	0.8
Layer hen	63	20	26.8	0.3	28.2	0.5	19.1	0.3	67.0	0.8	12.2	0.2	24.3	0.2
Layer hen	68	20	28.3	0.4	29.2	0.6	20.0	0.5	69.0	1.0	12.4	0.2	26.1	0.3
Layer hen	70	10	27.4	0.6	27.8	0.3	19.1	0.3	67.5	1.3	12.5	0.4	25.7	0.4
Layer hen	203	20	35.7	0.5	39.4	0.9	22.7	0.5	84.9	1.5	13.3	0.2	33.4	0.4
Layer hen	217	20	32.5	0.2	33.6	0.6	22.5	0.3	78.1	1.4	14.3	0.3	30.2	0.7
Layer hen	245	20	31.6	1.1	33.4	0.6	22.5	0.3	80.2	0.9	14.2	0.3	29.7	0.4
Turkey	78	8	36.9	0.7	42.5	0.6	34.0	0.7	104.6	1.8	20.3	0.6	34.7	0.8

3.3 Device designs

3.3.1 Modified Rabbit Zinger

The Rabbit Zinger^{TM} (Pizzurro, 2009a; Pizzurro, 2009b) is a penetrating captive bolt device originally designed to kill rabbits. It uses the stored energy in rubber tubes to drive a penetrating bolt into the top of the head, causing death by extensive irreversible brain damage (Figure 3.2). The device was modified with permission of the original inventor in order to adapt it to the new target species (i.e. poultry) as well as following observations of its use in a DEFRA funded trial in poultry (DEFRA, 2014), however the original function and bolt mechanism of the device was retained. The blue Power Tubes^{TM} (Pizzurro, 2009a) were used, which require 177 N to pull the bolt into the cocked position (Sparrey et al., 2014) and when fired the bolt delivered a mean of 11.87 J of kinetic energy (calculated from the mean speed (m/s) of 42 shots). Concerns over the power of the shot to concuss poultry have been raised (Hewitt, 2000), however more recent work conducted through a DEFRA trial demonstrated that it had potential to concuss poultry but required modifications to be more reliable and successful (DEFRA, 2014). The bolt measured 0.6 mm in diameter and was a smooth convex shape, with rounded edges. The bolt protrudes from the muzzle by approximately 3.5 cm. The device weighed 0.7 kg and measured 35 cm in length (un-cocked), increasing to 50 cm in length when cocked.

The device was operated by pulling the metal handle at the top of the bolt upwards, which results in the rubber tubes being stretched until the trigger pin sits within the pin catch (underneath the trigger), setting the device into the cocked state. Once the device is cocked, the animal is horizontally positioned on its ventral side onto a hard surface (e.g. table) and restrained, using the operator’s non-dominant hand. The operator’s dominant hand placed the device on top of the bird’s head, resting the muzzle between the ears and behind the comb, while gently applying pressure onto the head, so that the head was secured and rested against the horizontal hard surface. The device was fired by pushing the trigger inwards with the operator’s index finger. When firing the gentle downward pressure on the animal’s head was maintained in order to reduce recoil. The device operator always wore safety goggles and leather gloves.

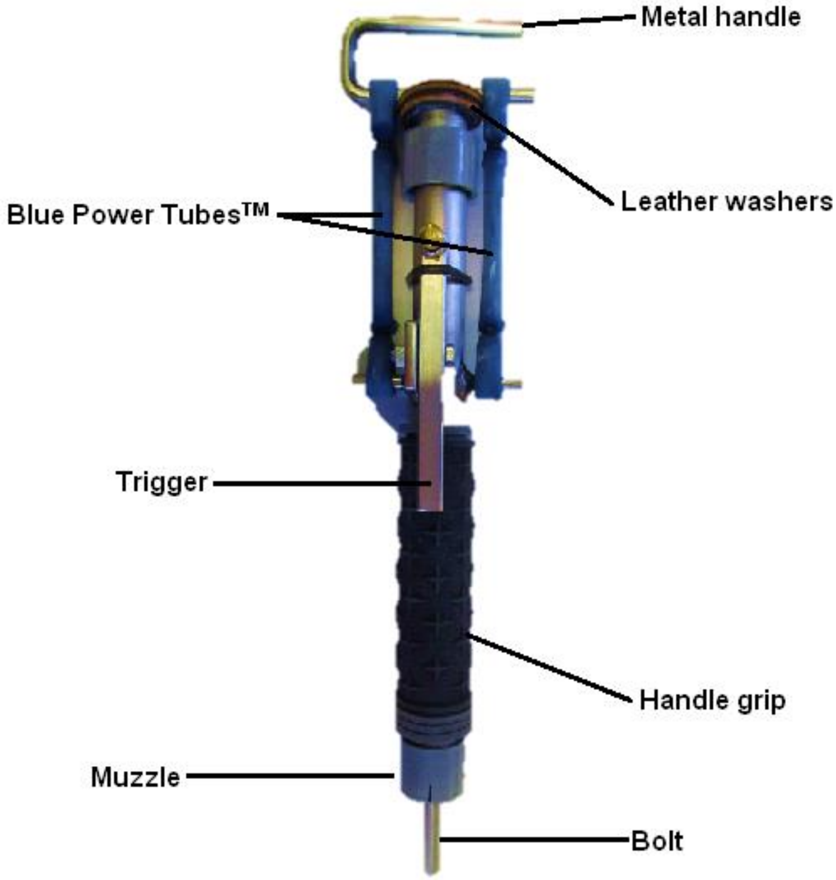


Figure 3.2 Diagram of the Rabbit Zinger™ with major components of the device labelled.

The modifications undertaken as part of this project consisted of three aluminium appendages added to the base of the device in order to secure the bird's head in place between them: two rested either side of the bird's head (over the ears, or auricular feathers) and the third ran down the front of the bird's face between the eyes and over the nostrils and beak. In the original modifications (Experiment 1 – Chapter 4) appendages were designed to position the bolt over the top of the bird's head in order to direct the bolt into the bird's hind and mid brain area from a slight ventral angle (Pizzurro, 2009b). Figure 3.3 displays photographs of the modified Zinger.

Following the results of Experiment 1 (Chapter 4.3) the shot angle was altered, with the muzzle resting further back on the skull and angled towards the beak (Figure 3.4). Additional leather washers were added to the bolt, in order to reduce the penetration depth from approximately 3.5 cm to 2.5 cm. The device was also weighted at the bottom in order to counteract the top heaviness of the device when cocked. The final modification was to change the bolt from a smooth convex shape, to a blunt edged concave shape, in order to reduce the risk of the bolt slipping off-target. To achieve this, the end of the bolt was bored into, causing an indentation of approximately 0.2 mm. Figure 3.5 shows a modified photograph demonstrating the area of trauma in relation to the size of the bolt.

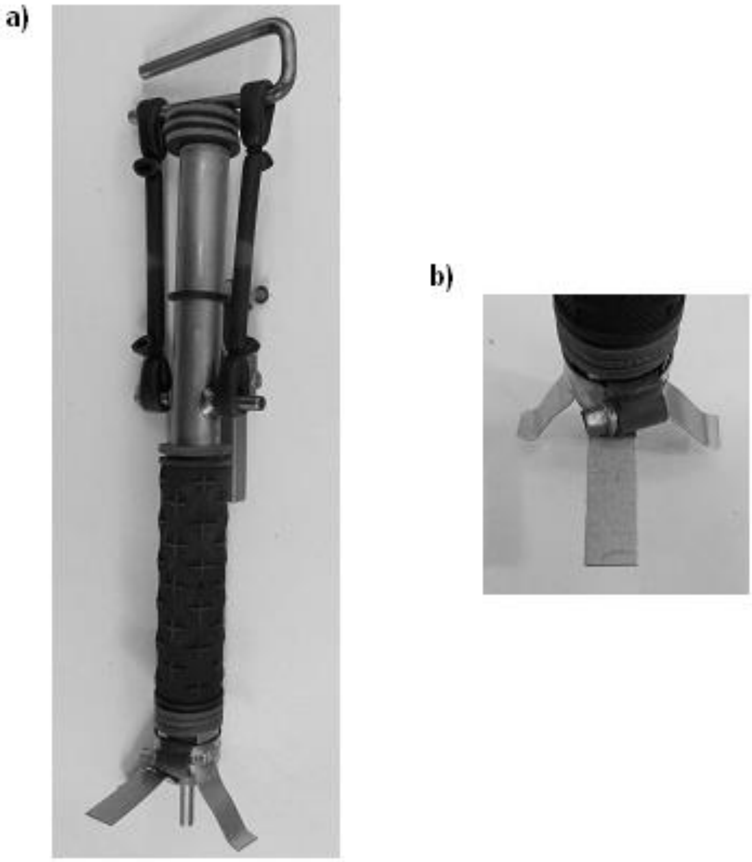


Figure 3.3 Photographs of (a) the modified Rabbit Zinger (MZIN) (un-cocked); and (b) a close-up image of the aluminium appendages added to the muzzle.

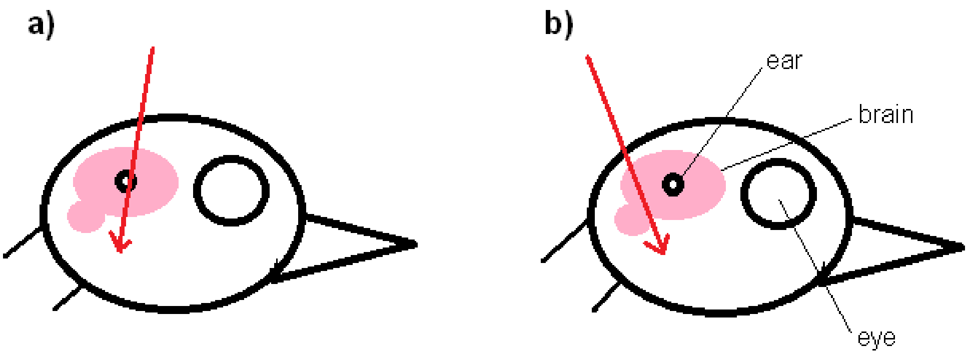


Figure 3.4 Diagrams demonstrating the angle of zinger shots (a) in original modifications – prior to experiment 1; and (b) following further refinement post Experiment 1.

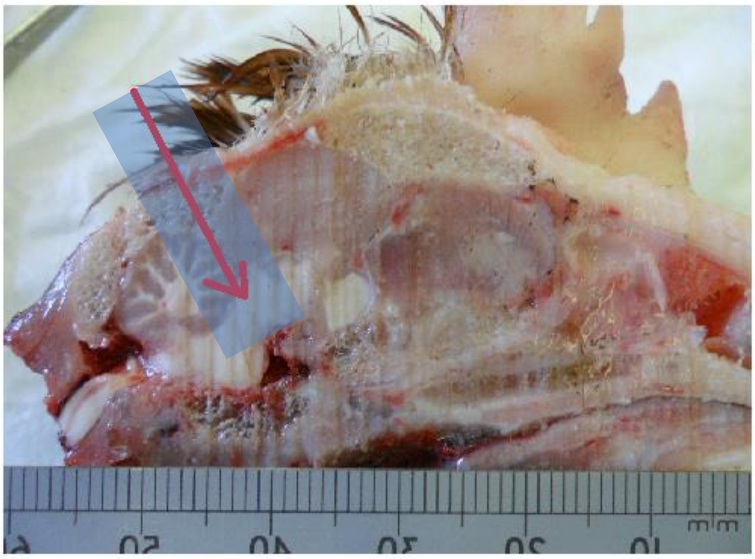


Figure 3.5 A scaled photograph of the bolt path drawn onto a cranial-caudal sagittal section of a chicken's head. The blue shaded area represents the scaled size of the bolt and the area of the head (and brain) which would be directly damaged following further refinement after Experiment 1 (Chapter 4).

The bolt trigger mechanism were not modified and all operating procedures stipulated, as well as maintenance in the Terms of Sale, were adhered to (Pizzurro, 2009a; Pizzurro, 2009b). The Power Tubes™ were replaced approximately after 50 shots and all moving parts of the device were cleaned and lubricated with White Mineral Oil® (Pure White Mineral Oil (Food Safe), Brandon Bespoke, Hampshire, UK).

3.3.2 Modified Armadillo

There is currently no established on-farm killing device for poultry which kills by penetration, like the puntilla device for cattle and llamas (Dembo, 1894; Limon et al., 2009; Limon et al., 2010; Limon et al., 2012); pithing in rodents and cattle (Close et al., 2007); and spiking in fish (Morzel et al., 2002; Robb et al., 2000). All of these devices work on the basis that if the spinal cord is severed from the brain within the area of the occipitoatlantal junction, it should render the animal immediately unconscious (Blackmore et al., 1995; Dembo, 1894). However, more recent research has disproved this theory by observing rhythmic breathing and cognitive responses post-application in several mammalian livestock species following puntilla-like methods, leading to suggestions that they are not humane (Blackmore et al., 1995; Dembo, 1894; Limon et al., 2009; Limon et al., 2010; Limon et al., 2012; Tidswell et al., 1987). However, research into the spiking of fish has suggested the method is humane (Robb et al., 2000; van de Vis et al., 2001), therefore there may be potential in such a method for poultry.

The Armadillo® is a brain-stem penetrating device designed by a vet (J Dalton) to dispatch game birds in the field (Figure 3.6), retailing at approximately £28. The device is a scissor-type mechanism, which involves the bird’s head being placed into the ‘cup’ of the lower arm (beak facing downwards) and when ready to apply the operator squeezes the handles together, which pushes the top arm (and the penetrating spike) downwards into the back of the bird’s skull, preferably through the foramen magnum therefore severing the top of the spinal cord (or brain stem), as well as causing generalised brain damage through cerebral hemorrhaging and changes in intracranial pressure (Freeman and Wright, 1953; Solomon, 1990; Whittow, 2000). As a result, the bird should die due to cerebral ischemia and/or massive damage to the base of the brain stem (medulla oblongata), resulting in cessation of respiration and blood circulation (Dunham et al., 2012; Kushner, 1998; Limon et al., 2010; Widjicks, 1995). The position and size of the cup was thoroughly investigated by the designer in order to consistently position the bird’s head at the appropriate angle for the spike to penetrate through the back of the head in the correct place (J Dalton, personal communication), however previous development was focused on its use in game birds and not poultry.

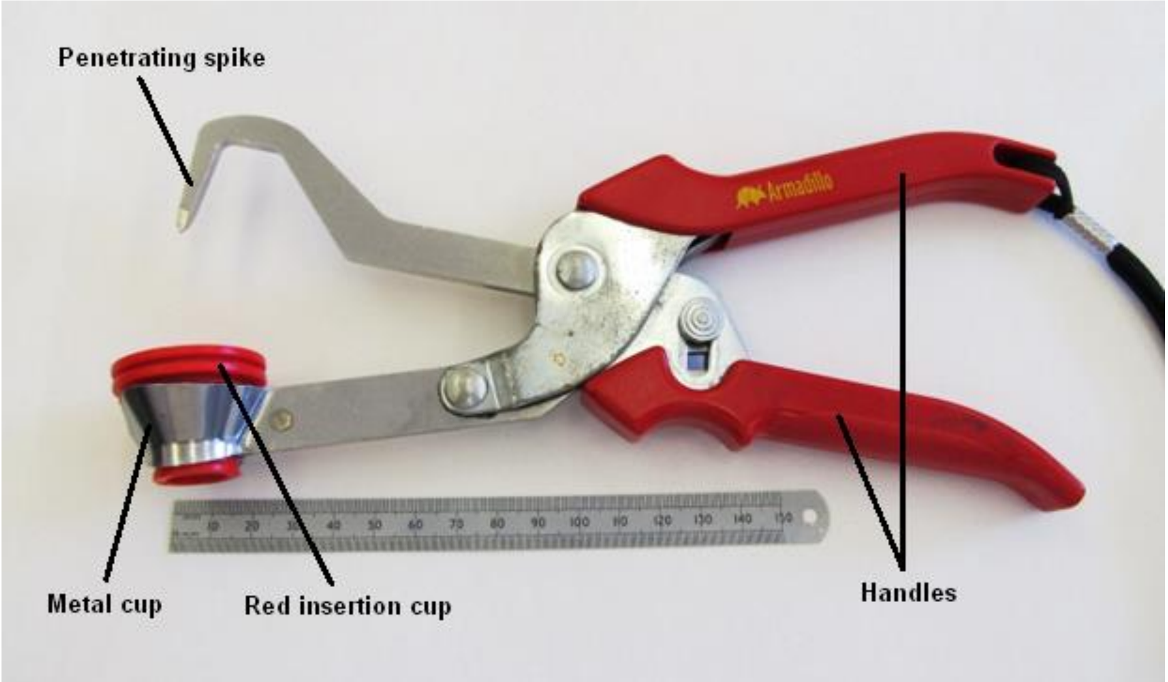


Figure 3.6 The Armadillo® (un-scaled image). The metal cup on the lower arm has a detachable red cup which can be inserted into the metal cup (in place in photograph) to adjust the size in relation to the target bird (e.g. pheasant or partridges)

Presently there is no published scientific evidence on the efficacy of this device, although it was evaluated on poultry in a DEFRA funded project (DEFRA, 2014), where it was shown to be inconsistent in its application and success rate. The DEFRA report also stated that there were issues with the device correctly fitting the different poultry types (turkey, broiler or layer hen) and production ages (chick to adult), despite the use of the additional red cup. As the device was designed to fit game birds, which are smaller in size and weight, it is understandable that there would be issues with the device fitting poultry, not only in terms of the bird’s face fitting into the cup, but also the depth and penetration site of the spike.

Due to the promising simplicity of the Armadillo® device, which would not rely on the operator’s strength or skill for correct application, it was selected as a device to modify in order to accurately fit primary poultry species (e.g. broilers and layer hens and turkeys at various stages of production. However, turkeys were not tested in this project. Modifications consisted of replacing the lower arm of the device (Figure 3.7) and increasing the upper (33 mm to 37 mm) and lower (19 mm to 27 mm) diameters of the openings of the metal cup (Figure 3.8). Three additional green insertion cups were molded from 1 mm thick plastic funnels, in order to generate multiple adjustments to fit the various sizes of birds' heads, as demonstrated as being potentially important in the pilot work (Section 3.2), as well as to position and restrain the bird's head correctly in order to produce sufficient penetration of the spike into the bird's head. The green cups also had soft padding (Waxman 4719095N ½ inch Self Stick Felt Pads, Waxman, Ohio, United States) added to their sides, which would cushion the lateral sides of the bird's head (over the eyes) as well as creating an oval shape for the upper opening (Figure 3.9). The three sizes of green cups were labelled: G1, G2 and G3 and their dimensions are listed in Table 3.2.

Figure 3.7 Photograph of the modified Armadillo® (MARM) showing the larger metal cup.

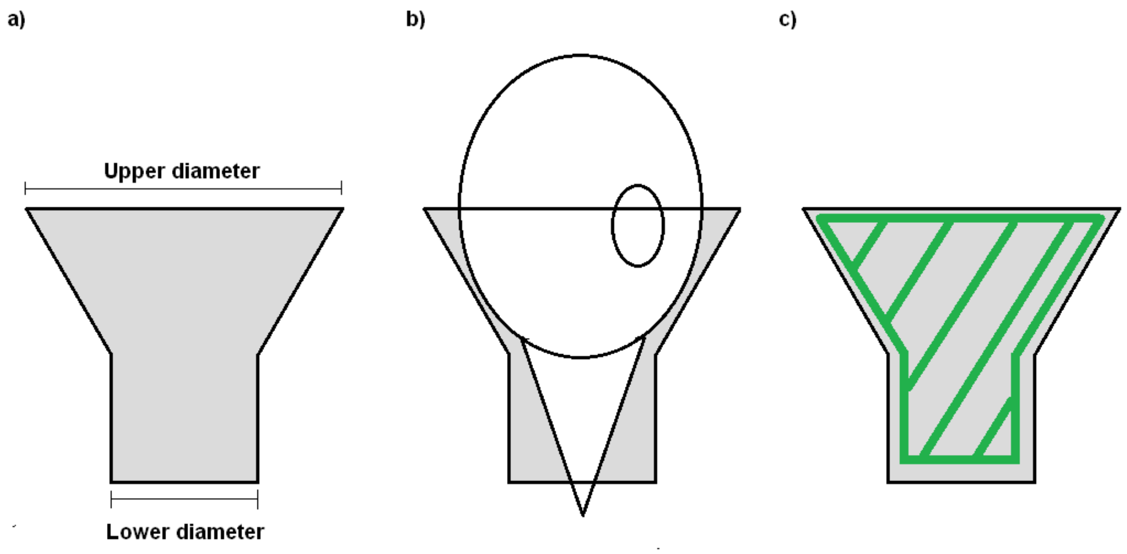


Figure 3.8 Diagrams of (a) the basic metal cup attached to the lower arm of the Armadillo®, with the upper and lower openings identified; (b) representation of a bird's head within the metal cup; and (c) the addition of a green cup to the metal cup to adjust to size of bird. Refer to Table 3.2 for dimensions.

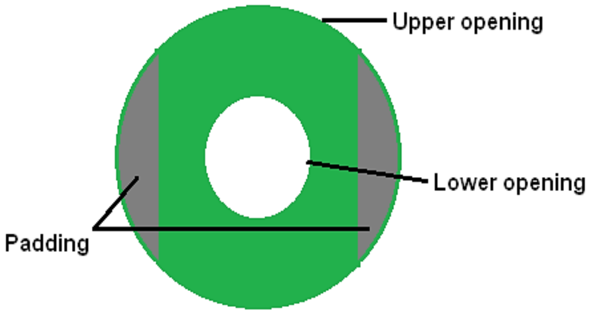


Figure 3.9 Diagram of a green cup, displaying the location of the padding.

Table 3.2 Dimensions (mm) of the three green insertion cups and their internal padding, as well as the proposed suitable bird types.

Green cup	Upper diameter	Lower diameter	Distance between padding	Size (L x W x D) of padding	Proposed bird type use
G1	41	27	38	35.0 x 15.0 x 1.5	Slaughter- age turkey
G2	36	23	33	20.0 x 10.0 x 1.5	Slaughter-age broilers and end-of-lay hens
G3	30	18	27	10.0 x 7.0 x 1.5	Layer pullets and broiler chicks

3.3.3 Modified Pliers

Cervical dislocation pliers were reported as the second most popular killing method for poultry on-farm in the UK, (see Chapter 2.4) and a review by Sparrey et al. (2014) also reported the popularity of the device with small poultry keepers in the UK. There are several forms of dislocating pliers on the market (e.g. Semark pliers, turkey neck pliers (HSA, 2004)), however, research has demonstrated they do not cause an immediate loss of consciousness (e.g. loss of Visual Evoked Responses (VERs) as a possible indicator of loss of consciousness (DEFRA, 2000; Gregory and Wotton, 1990)), and in particular for the Semark pliers there is a low success rate in fully dislocating the neck and severing the spinal cord (Gregory and Wotton, 1990). There was little evidence that plier devices resulted in carotid arteries being severed and the physiological trauma produced was consistent with crushing injuries (DEFRA, 2000; Gregory and Wotton, 1990). Any killing method which causes crushing injury is no longer permitted under the new European Council Regulations on the Protection of Animals at the Time of Killing (European Council, 2009).

Due to the confirmed popularity of this type of killing method and the simplicity of its mechanical design, which like the MARM reduces the reliance on the operators skill and strength, it was decided to attempt to modify cervical dislocation pliers, in order to prevent crushing injury, as well as increase the success rate and humaneness (e.g. reduce time to loss of consciousness). Semark pliers (Maun Industries Ltd., Nottingham, UK - Figure 3.10) were selected to be modified as they have in the past been marketed for despatching chickens as well as other poultry species and are a small single-handed device. The device weighs approximately 200 g and has an overall length of 180 mm. When the blades of the device are fully open the maximum distance between the upper and lower teeth is 36 mm. When the blades are fully closed there is a slight gap between the blades (< 1 mm).

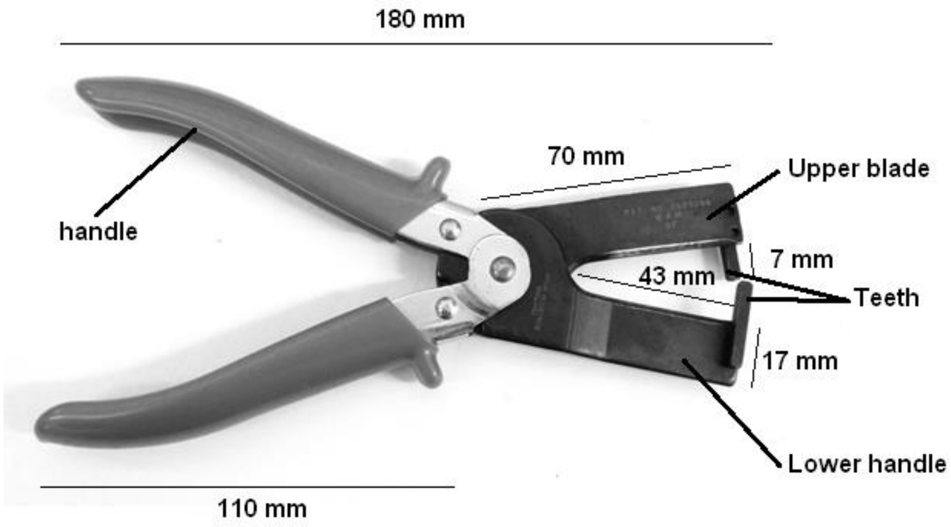


Figure 3.10 Semark pliers showing various dimensions.

The only modification made to the device was to change the shape and width of the blades in order to create a narrower, curved blunt edge rather than a straight blunt edge (Figure 3.11). The edges of the blades remained blunt in order to reduce the risk of skin breakage and thus blood loss during application of the method. It was hypothesised that by narrowing the edge of the blade it would reduce the risk of crushing and would also increase the likelihood of dislocation, as the narrower blade would more easily slip between two cervical vertebra when force was applied (Figure 3.12). The blades were curved in order to gradually increase the size of the blade and therefore generate a dislocation (i.e. gap between the two vertebra), through pushing the vertebrae apart. Other features such as the size of the device, including the length of the blades and the presence of “teeth” at the ends of the blades, were not altered, as the range of neck sizes measured during the pilot work were all smaller than the length of the blades as well as being smaller than the maximum gap between the upper and lower blade teeth. The modified device was termed Modified Pliers (MPLI).

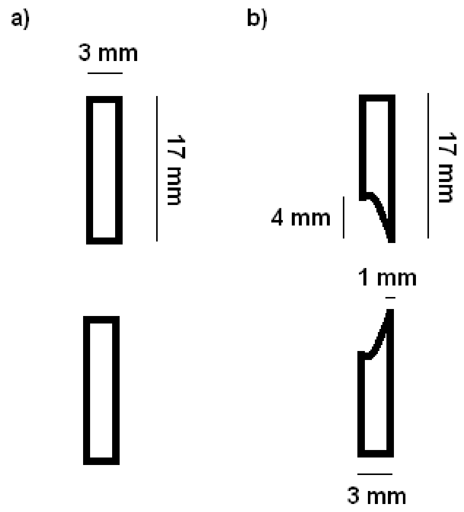


Figure 3.11 Comparison of (a) the un-modified Semark plier blades and (b) the MPLI blades, with dimensions.

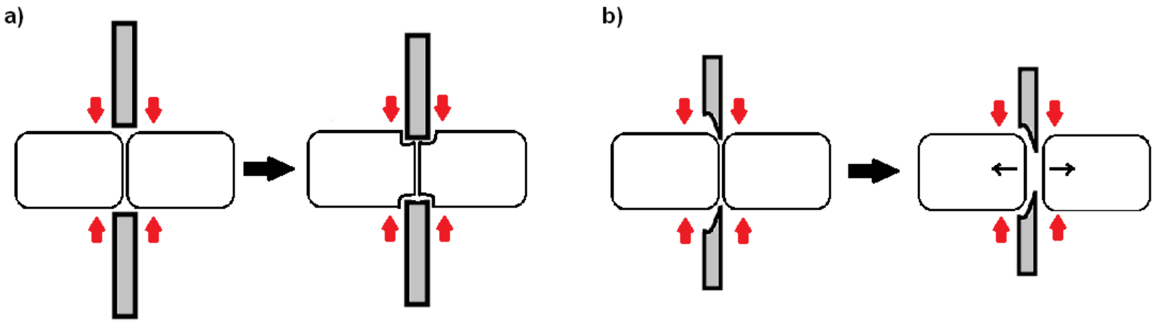


Figure 3.12 Hypothesised effect of (a) original Semark pliers; (b) the modified Semark pliers on stylised cervical vertebrae.

3.3.4 Novel Mechanical Cervical Dislocation Device

In response to the manual method's high popularity and application concerns it was decided to develop a tool to aid the application of manual cervical dislocation, in essence making the method mechanical, in an attempt to improve its consistency in application, as well as its humaneness.

The basic design was to create a glove device to replicate the action of MCD (refer to Chapter 1.4.1). The device consisted of a supportive glove (SHOWA 370 Multi-purpose

Stable Glove™, UK) designed to support the operator's wrist and hand (and therefore could reduce strain injury in the operator) and a moveable metal insert. The metal insert fingers (LH and RH - Figure 3.13) were designed to fit around the bird's head to create a secure grip, and when overlapped at their base and screwed together, the ability to move independently from side to side in order to allow adjustment for different sizes of birds (Figure 3.14). The maximum sized bird (B $\leq$ 36 mm (refer to Section 3.2)) on which the device could be used was a bird weighing < 5 kg (i.e. chickens (including breeding stock) and small turkeys). This weight restriction also conforms to the European Regulations 1099/2009 (European Council, 2009) on mechanical dislocation. The rounded shape of the metal fingers was designed to aid the twisting motion required to dislocate the bird's neck by enhancing the "rolling action" of the hand. The blunt edge between the two metal fingers provided a hard edge to force between the back of the bird's head and the top of the neck, designed to focalise the force into the desired area (i.e. a dislocation at C0-C1) when the method was applied.

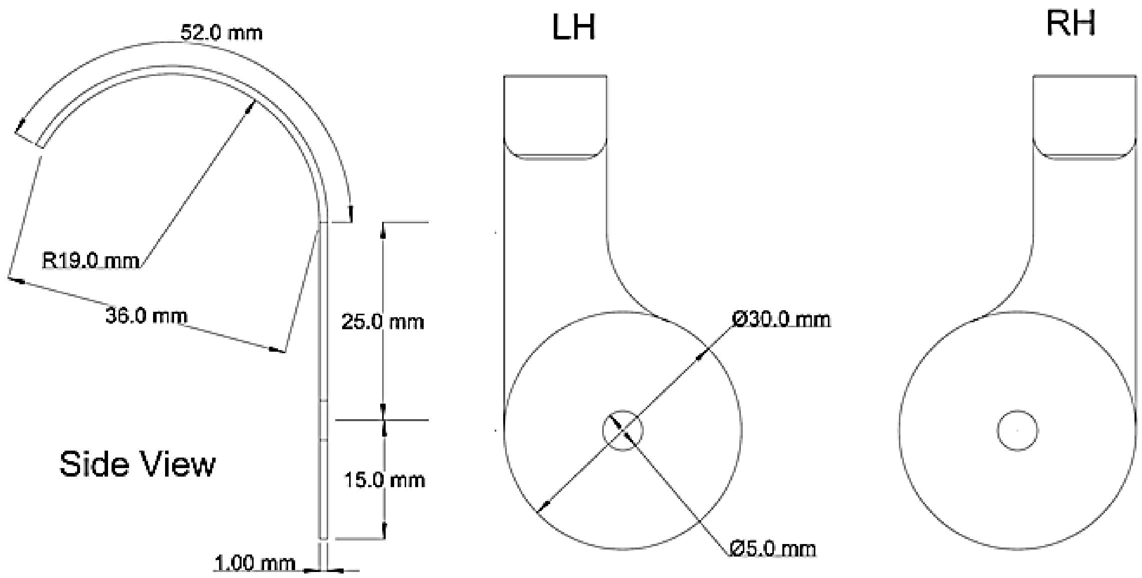


Figure 3.13 Diagrams and dimensions of the metal finger inserts, showing side, left-hand (LH) and right-hand views. The fingers were constructed from 2.5 mm thick aluminium. Schematic drawings provided courtesy of Julian Sparrey.

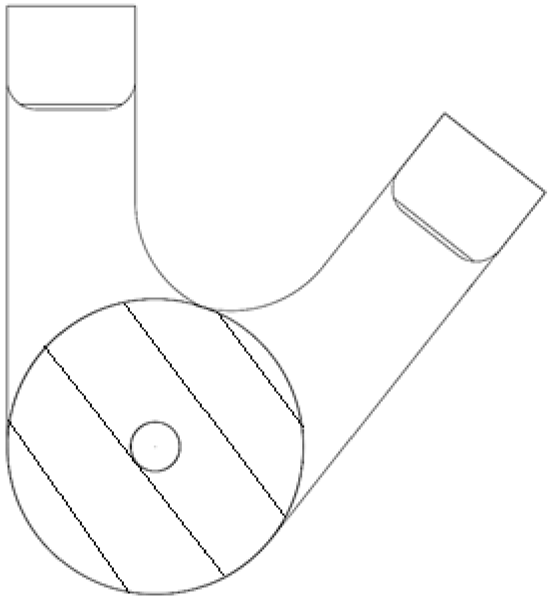


Figure 3.14 Diagram of the combined LH and RH metal fingers, Secured together by a hinge joint through a pin and locking cap, therefore allowing the fingers to move independently. The pin cap was covered with a padded leather washer (Leather washers, 3cm, RH Nuttall Limited, Birmingham, UK), padding the area which the bird's head sits in. Schematic drawing provided courtesy of Julian Sparrey.

Figure 3.15 displays the final product; termed the Novel Mechanical Cervical Dislocation method (NMCD). It was hypothesised that the device could be made to fit different operators by inserting the metal inserts into different sizes of glove (i.e. small/medium/large). For this project the operator wore a size small (S) glove. The glove device was worn on the hand which holds the bird's head. The device was designed to be tight fitting in order to maintain relatively strong tactile sensation for the operator through the glove, in order to correctly adjust the metal fingers where necessary. The operator's index and middle fingers rested above the finger inserts and the hinge joint sat on the fleshy pad below the fingers. The tips of the metal fingers rested under the bird's jaw and the metal hinge joint rested behind the bird's skull, at the top of the neck. The operator secured the bird's head in place by placing their thumb and ring finger under the bird's chin. The operator's un-gloved hand was used to hold the bird's legs (securing the bird upside down), resting its underside against the operator's thigh. The device was applied in one swift movement with the gloved hand pulling downwards on the head, while also rotating the head back towards the ceiling and forcing the metal edge into the back of the bird's head and the top of the neck (Figure 3.16).

Figure 3.15 The completed NMCD device: metal inserts in situ within the glove. The metal inserts were secured with Velcro® (Velcro VEL-EC60214 20mm x 2.5m Brand Stick on Tape, Velcro Industries, UK) within the glove.

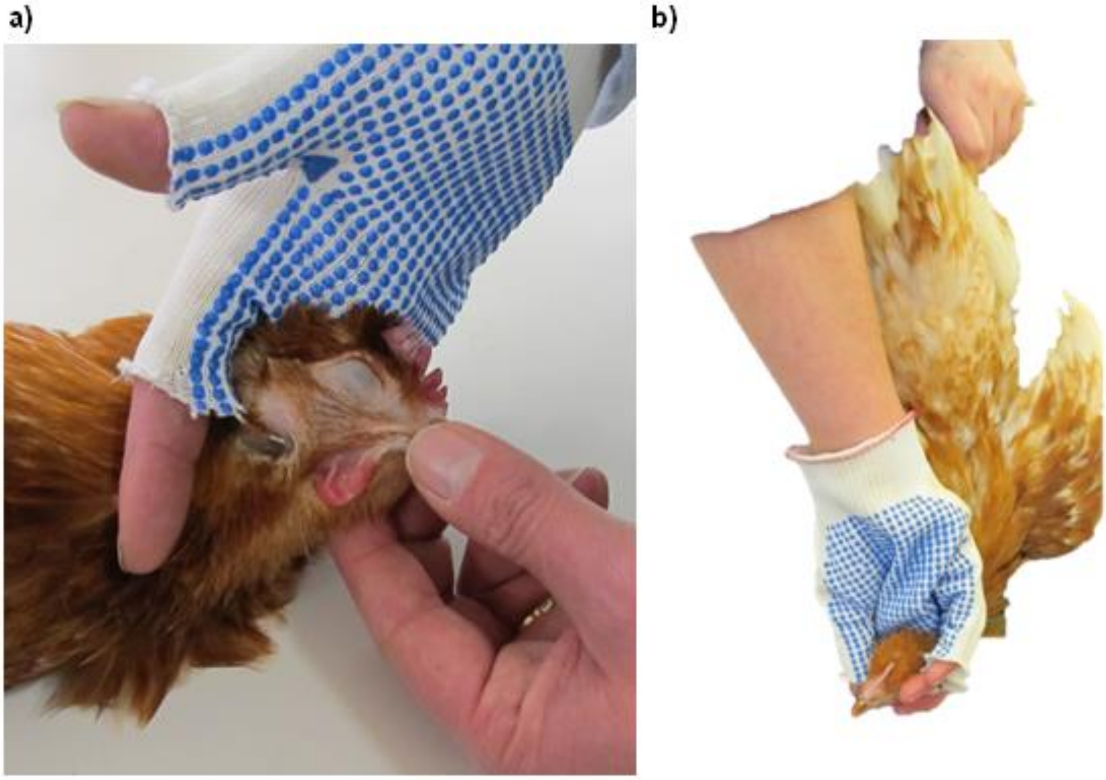


Figure 3.16 (a) the NMCD glove positioned on the head of a 12 week old layer pullet cadaver; and (b) the device in position on the same cadaver in which the neck is dislocated.

3.4 Manual Cervical dislocation (MCD)

MCD was used a control treatment for experiments 2, 3 and 4. It was performed following the HSA's guidelines; with the bird held upside down by both legs in one hand, and the bird's head held in the operator's palm with the neck between the index and middle finger of the other hand (HSA, 2004). In one swift movement, the operator pulled down on the bird's head, stretching the neck, while rotating the bird's head upwards towards the back of the neck.

3.5 Ethical Statement

This project and its four experiments were performed under UK Home Office licence authority via Project and Personal licences and underwent review and approval by SRUC's ethical review body. All routine animal management procedures were adhered to by trained staff.

4 Testing the efficacy of mechanical killing devices on broiler and layer cadavers

4.1 Introduction

Determining the success rate of a killing device is essential to evaluating its overall efficacy. The designing and prototyping of novel and modified devices is the first stage in their development, the next stage is to assess the devices' performance on the desired target species. This study is the first part of a four stage project to design and evaluate alternative mechanical methods for killing poultry on farm, which conform to the new legislation (European Council, 2009) on the Protection of Animals at the of Killing. The newly designed devices need to be inexpensive, simple to use, easily maintained, portable and effective. If a device does not meet most or all these criteria, it will not be successfully adopted by industry or hobby poultry keepers in the future.

It is paramount to ascertain that the devices cause sufficient trauma to the birds' anatomy to result in rapid loss of consciousness and death. Previous research has shown that post-mortem analysis is effective in inferring killing potential and time to loss of consciousness and has been used across several species in determining success rates of slaughter and on-farm killing method in livestock species (Anil et al., 2002a; Bader et al., 2014; Grandin, 2010; Gregory, 1994; Morzel et al., 2002; van de Vis et al., 2001). For example the successful application of cervical dislocation methods is determined by the animal having its neck dislocated and the spinal cord severed (Bader et al., 2014; Carbone et al., 2012; Cartner et al., 2007; Erasmus et al., 2010a), while for concussive (head trauma) devices, there must be sufficient damage (e.g. skull fractures, brain contusions, cerebral oedema, hemorrhaging and *contra-coup* damage) (Finnie et al., 2000; Finnie et al., 2002; Gregory et al., 2007; Gregory and Shaw, 2000).

The survey conducted in Chapter 2 highlighted key preferences for on-farm killing devices in poultry and as a result four mechanical devices were designed and prototyped: the Modified Armadillo - MARM; Modified Rabbit Zinger - MZIN; Modified Pliers - MPLI; and a Novel Mechanical Cervical Dislocation Device - NMCD The aim of this study was to ascertain if the devices caused sufficient anatomical trauma to cadaver birds, which could be inferred to be their killing potential, as well as evaluating if the devices were performing in their specific designed and hypothesised way.

4.2 Methods and materials

4.2.1 Animal Housing

The experiments were conducted between March 2012 and June 2012. A total of 160 female layer-type and meat-type chickens were used for the study across four batches and distributed across two types and ages (Table 4.1). Birds were collected from commercial farms and transported to SRUC facilities in four batches; 40 birds per batch, with each batch containing the four bird type + age combinations. All birds were weighed and wing-tagged on arrival.

The birds were housed for one week prior to the experiment in order to allow them to acclimatise to the new environment. Birds were housed in separate rooms per bird type and age group to provide recommended environmental controls (Aviagen, 2009; Hy-Line, 2012). All birds were kept in floor pens with wood-shavings litter at lower than commercial stocking density and with suitable environmental enrichments (DEFRA, 2002a; DEFRA, 2002b). All pens were constructed from wooden frames with wire-grid sides and roof, allowing visual and auditory contact with other birds within the same room. Broiler chicks and layer pullets were housed in group pens (L 1.5 m x W 2.5 m x H 1.5 m). Broilers (slaughter-age) and layer hens were kept in pairs. Pen sizes were L 1.5 m x W 0.5 m x H 1.5 m. All birds had *ad libitum* access to appropriate food and water. All birds were inspected twice daily, and the minimum and maximum temperatures were recorded each morning.

Table 4.1 Accommodation and bird details for each bird type and age group.

Bird group	N	Mean bird age at killing (days)	Mean bird weight at killing (kg)	N per pen	Pen furniture
Layer pullets (Lohmann strain)	40	73.5 ± 0.2	0.8 ± 0.1	10	2 x feeder, 4 automatic cup drinkers, 2 x wooden perch, 2 x nest box, 4 x suspended blue string
Layer hens (Lohmann strain)	40	487.9 ± 0.9	1.8 ± 0.1	2	1 feeder, 2 automatic cup drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string
Broiler chicks (Ross 308 strain)	40	22.43 ± 0.1	0.7 ± 0.2	10	2 x feeder, 1 x automatic large bell drinker, 4 x suspended shiny objects
Broiler (slaughter age) (Ross 308 strain)	40	37.1 ± 0.6	1.9 ± 0.7	2	1 feeder, 2 automatic cup drinkers, 2 x suspended shiny objects

4.2.2 Study Design

Four mechanical poultry killing devices, the MARM, MZIN, MPLI and NMCD were assessed for their killing potential in cadaver birds. The device designs are described in detail in Chapter 3.3. The experiment was designed around a 4 x 4 x 4 factorial design (batch x device x bird type + age). 10 birds per bird type (+ age) were tested with each of the four mechanical devices (N = 160 birds). Birds were tested in four one week batches, with birds being tested in blocks of ten. A Graeco Latin square was used to balance batch, block, bird type (+ age) and device. Within this, 4 Latin squares (1 per batch) were used to balance block, test order in block and bird type (+age), with the test order in each block then repeated until all 10 birds are tested.

The birds were humanely euthanised by an intravenous sodium pentobarbital injection (Euthatal, Merial Animal Health Ltd., Essex, UK) immediately prior to device testing in order to maintain cadaver freshness and minimise blood coagulation.

4.2.2.1 Post mortem evaluation

After device application, the cadavers were immediately examined post-mortem in order to establish as accurately as possible the anatomical damage sustained to the bird by the device. All cadavers were weighed prior to testing and schematic measurements of the head and neck were taken (refer to Chapter 3 - Figure 3.1). Specific post-mortem measures were recorded for each killing treatment as their target areas were different. For all killing treatments binary yes/no measures were recorded for skin broken, external blood loss and subcutaneous hematoma and the total number of attempts were recorded (e.g. multiple pulls for NMCD or miss-fire of MZIN).

For the MZIN and MARM, seven specific measures were recorded: binary yes/no measures of damage to the skull, specific brain regions (left forebrain, right forebrain, cerebellum, midbrain and brainstem); and the presence of an internal brain cavity hematoma.

For killing treatments which caused trauma to the neck of the bird, seven specific post-mortem measures were assessed: four binary measures (yes/no) were recorded for dislocation of the neck, vertebra damage (e.g. intra-vertebra dislocation/break), damage to neck muscle, crushing injury to the trachea or oesophagus and whether the spinal cord was severed. The level of cervical dislocation was recorded (e.g. between C0-C1, C1-C2, C2-C3, etc.), as well as a measurement of the length (cm) of gap between the dislocated cervical vertebra. The number of carotid arteries severed was also recorded as either zero, one or both.

4.2.2.2 Derived kill potential and device success

From the post-mortem evaluations two binary (yes/no) measures were derived: kill potential and device success. Kill potential was defined as the cadaver exhibiting sufficient damage to the anatomy which would have resulted in death (if the bird had been alive at testing) following one attempt. For example, this was confirmed dislocation of the neck and severing of the spinal cord for NMCD and MPLI (Bader et al., 2014; Erasmus et al., 2010a; Gregory and Wotton, 1990); and diffuse brain damage for the MARM and MZIN (Finnie, 1993; Finnie et al., 2000; Finnie et al., 2002; Limon et al., 2010).

Device success was defined as when the device caused the desired anatomical damage, dictated by its hypothesised design, as well as producing sufficient damage which would have resulted in death (if the bird had been alive at testing) and based on scientific literature would be most likely to minimise time to unconsciousness post device application. Device success criteria were device specific and are described in Table 4.2.

Table 4.2 Defined device success parameters for each killing device.

Device	Device success criteria	References
MARM	• Spike penetrates through foramen magnum of the skull • Severing of brain stem	(Dembo, 1894; Limon et al., 2009; Limon et al., 2010; Limon et al., 2012)
MZIN	• Skull is penetrated and damaged • Severe damage to a minimum of one area of the brain	(Erasmus et al., 2010a; Finnie, 1993; Finnie et al., 2002; Gregory et al., 2007; Pizzurro, 2009b)
MPLI	• Complete cervical dislocation at C0-C1 • Severing of the top of the spinal cord (i.e. brain stem) • Severing of both carotid arteries • No breakage to the skin • No crushing injury to the trachea or oesophagus	(Bader et al., 2014; Dimar et al., 1999; Dumont et al., 2001a; Erasmus et al., 2010a; Gregory and Wotton, 1990; HSA, 2004; Sparrey et al., 2014; Tidswell et al., 1987; Weir et al., 2002)
NMCD	• Complete cervical dislocation at C0-C1 • Severing of the top of the spinal cord (i.e. brain stem) • Severing of both carotid arteries • No breakage to the skin	(Bader et al., 2014; DEFRA, 2014; Dimar et al., 1999; Dumont et al., 2001a; Erasmus et al., 2010a; Gregory and Wotton, 1990; HSA, 2004; Sparrey et al., 2014; Tidswell et al., 1987; Weir et al., 2002)

4.2.3 Statistical Analysis

All data collected at the bird level were summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat (14th Edition). Statistical significance was termed by a threshold of 5% probability based on F tests. Summary graphs and statistics were produced at the bird level. For all models, batch was used as the random model. All fixed effects were treated as factors and classed as categorical classifications.

4.2.3.1 Post-mortem evaluations

Data was subset twice, initially to remove unsuccessfully “killed” birds (i.e. kill potential “no”) in order to prevent data skewing; and then into two groups dependent on trauma area: 1) neck trauma (NMCD and MPLI); and (2) head trauma (MZIN and MARM), in order to allow logical comparison between killing treatments which damaged the neck or the head. Analysis of post-mortem binary measures (e.g. skin break, subcutaneous hematoma, etc.) and categorised measures (e.g. cervical dislocation level, number of carotid arteries severed, etc.) was conducted via Generalised Linear Mixed Models (GLMMs) using logit link function and binomial distribution. Fixed effects included were killing treatment, bird type, bird age, and their interactions. For killing treatments which damaged the neck, some variables were also included as factors in modelling for other variables (e.g. variable = dislocation level, factor = neck gap size). For killing treatments which damaged the head, the variable of skull penetration (yes/no) was also included as a factor in modelling for the binary variables of brain regions damaged (e.g. brain stem, cerebellum, etc.).

4.2.3.2 Kill potential and device success

Statistical comparisons for kill potential and device success were conducted with GLMMs, using logit link function, and binomially distributed errors due to the nature of the binary data. In the maximal models, fixed effects included killing treatment, bird type, bird age, and all their interactions. Dispersion was fixed at one.

4.3 Results

Mean (±SE) bird weights and schematic measures of the head and neck are shown in Table 4.3.

Table 4.3 Mean (±SE) of bird weight and schematic measures of the head and neck at the time of killing across the two bird types (broiler/layer) and bird grouped ages. Refer to Chapter 3 - Figure 3.1.

Bird type and age	Bird weight (kg)	Head and neck schematic measures (mm)						
		A	B	D	E	F	G	N1
Broiler chicks	0.7±0.2	24.8±0.3	25.5±0.1	19.4±0.2	60.9±0.5	10.5±0.1	12.7±0.1	13.1±0.2
Broilers (slaughter age)	1.9±0.7	30.5±0.4	31.0±0.3	21.8±0.3	75.8±0.7	13.2±0.2	15.9±0.2	17.9±0.4
Layer pullets	0.8±0.1	27.2±0.3	28.0±0.2	18.6±0.3	68.6±0.8	12.3±0.1	13.4±0.2	13.6±0.2
Laying hens	1.8±0.1	31.2±0.3	31.8±0.2	21.2±0.2	78.0±1.8	13.8±0.1	15.3±0.2	17.3±0.3

4.3.1 Kill potential and device success

A total of 36 birds were not successfully “killed” on the first attempt (NMCD = 0/40 birds; MPLI = 15/40 birds; MARM = 15/40 birds; and MZIN = 6/40 birds). Killing method had an effect on kill potential (Table 4.4), with NMCD having the highest kill potential, with 100% of birds sustaining the required physiological trauma to have caused death (Figure 4.1). The MARM and MPLI had the lowest kill potential, with both achieving 62.5%. Bird age was the only other factor to affect kill potential (no = 0; yes =1), with younger birds being more likely to sustain the required physiological trauma to have caused death (mean = 0.87 ± 0.04), compared to older birds (mean = 0.68 ± 0.05). All other factors and their interactions had no effect on kill potential.

GLMM showed that device success was significantly affected by killing method (Table 4.4), with NMCD shown to be most likely to perform in the desired way and producing optimal damage to the birds (Figure 4.1). Like kill potential, bird age significantly affected device success, with younger birds (mean = 0.69 ± 0.05) being more likely to sustain optimal physiological damage compared to older birds (mean = 0.53 ± 0.06). All other factors and their interactions had no effect on device success.

Table 4.4 GLMM analysis output of the minimum models for effects on kill potential and device success. Significant *P* values are underlined.

Fixed effects	df	Kill potential		Device success	
		F statistic	*P value*	*F statistic*	*P value*
Killing method	3	2.88	<u>0.038</u>	7.00	<u><0.001</u>
Bird type	1	0.92	0.340	0.19	0.661
Bird age	1	5.15	<u>0.025</u>	5.03	<u>0.026</u>
Bird weight	1	0.48	0.771	0.00	0.996
Killing method. Bird type	3	0.13	0.943	0.44	0.728
Killing method. Bird age	3	0.42	0.737	0.15	0.931
Killing method. Bird weight	3	0.27	0.813	0.56	0.644

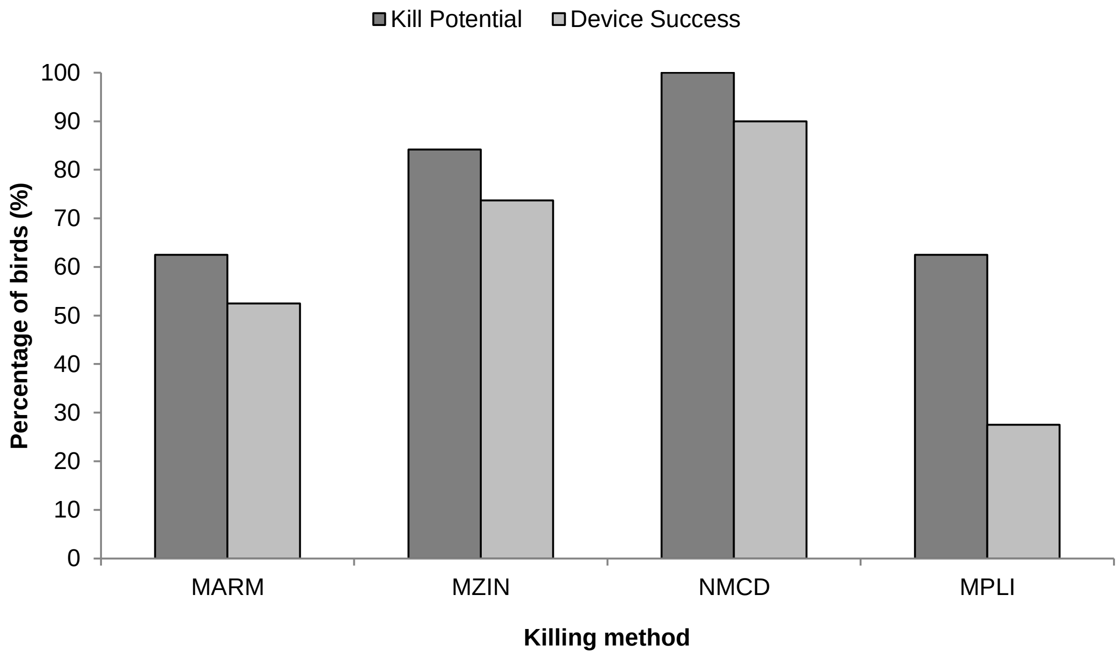


Figure 4.1 Summary of kill potential and device success rates (%) across the four killing treatments.

4.3.2 Post-mortem evaluations

4.3.2.1 Cervical dislocation methods: MPLI and NMCD

For successfully killed birds (MPLI = 15/40 birds; NMCD = 40/40 birds), the percentage of birds for which the relevant neck trauma post mortem factor was present, according to killing method is shown in Table 4.5. MPLI was more likely to tear the skin, cause external bleeding, vertebral damage, trachea damage, and oesophagus damage compared to NMCD, although the differences were not significant. NMCD was significantly more likely to cause a cervical dislocation, as well as severing one or more carotid arteries compared to MPLI (Figure 4.2). However, the location of the dislocation (e.g. C0-C1, C1-C2, etc.) was not significantly affected by killing method ($F_{3,159}$ = 2.34, P = 0.076), although it had a tendency (P < 0.10), with NMCD to be more likely to cause a higher level dislocation (e.g. C0-C1) compared to MPLI (Figure 4.3).

Table 4.5 Percentage of birds killed successfully for which the relevant neck trauma post mortem factor was present, according to killing method, including GLMM analysis of killing method comparison. Significant *P* values are underlined.

Post mortem measure	Percentage of birds		*F statistic*	*P value*
	NMCD	MPLI		
Skin broken	7.5	20.0	0.32	0.570
External bleeding	2.5	7.5	0.06	0.805
Subcutaneous hematoma	100.0	72.5	0.00	0.994
Cervical dislocation	100.0	45.0	11.86	<u><0.001</u>
Vertebral damage	5.0	55.0	3.26	0.071
≥1 carotid artery severed	95.0	15.0	6.34	<u>0.012</u>
Trachea damage	0.0	52.5	3.41	0.059
Oesophagus damage	0.0	12.5	0.13	0.870
Spinal cord severed	100.0	67.5	0.00	0.998

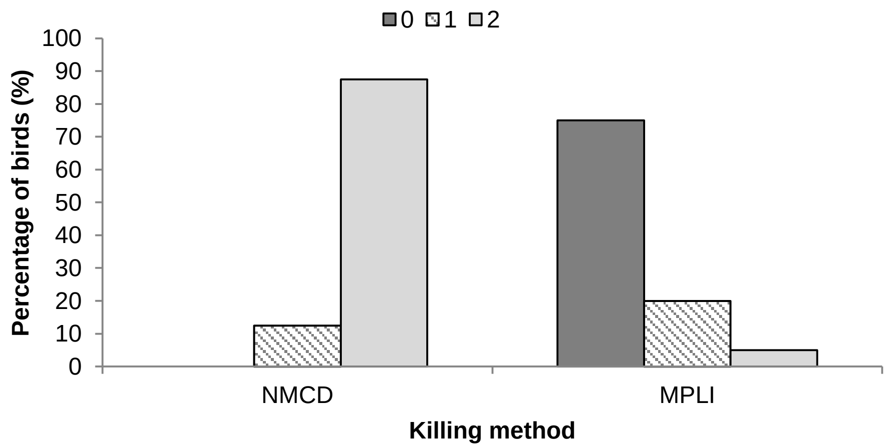


Figure 4.2 Distribution of birds across the number of carotid arteries severed dependent on killing method.

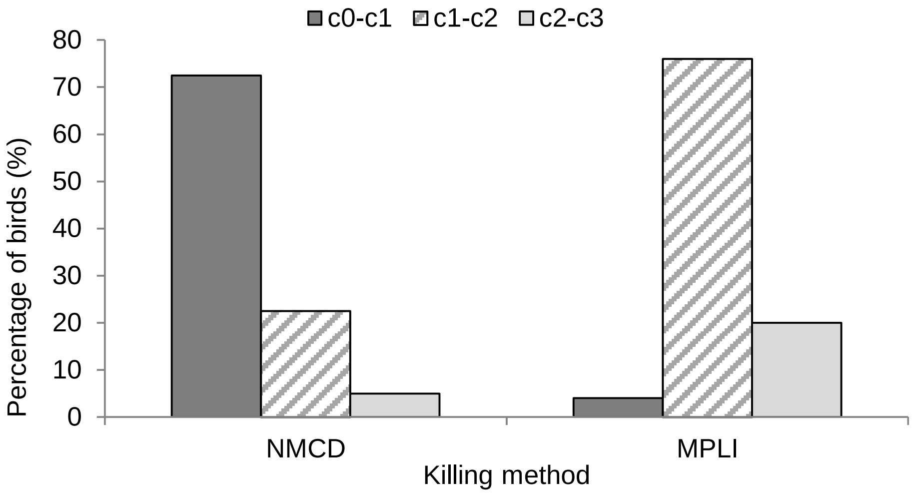


Figure 4.3 Distribution of birds across the various dislocation levels dependent on killing method.

Other factors such as bird type, bird age, and bird weight and their interactions with killing method had no effect on skin tearing, external bleeding, subcutaneous, hematoma, trachea damage, oesophagus damage, and dislocation level. However, for number of carotid arteries severed, vertebral damage, dislocation, and dislocation level, some other factors did have an effect (Table 4.6). The neck diameter of the birds (N1) had a tendency to affect the number of carotid arteries severed, with a significant negative correlation (r = -0.382, P = 0.047) between these.

Whether or not cervical dislocation (no = 0; yes = 1) occurred was significantly affected by bird type and bird age, with dislocations more likely to occur in broilers (mean = 0.95 ± 0.05) rather than layers (mean = 0.55 ± 0.11), and younger birds (mean = 0.90 ± 0.07) compared to older birds (mean = 0.60 ± 0.11). The N1 was also shown to have an effect with unsuccessful dislocations associated with larger neck diameters compared to smaller neck diameters (N1 means: no = 17.1 ± 1.09 mm; yes = 14.9 ± 0.51 mm).

Bird type had an effect on vertebral damage (no = 0; yes = 1), with layers (mean = 0.75 ± 0.10) more likely to sustain damage than broilers (mean = 0.35 ± 0.11). No other factors or interactions, apart from killing method (reported above) had an effect.

Table 4.6 GLMM output for post-mortem measures (number of carotid arteries severed, dislocation , vertebral damage, dislocation level).

Fixed effects	df	Number of carotid arteries severed		Dislocation occurred		Vertebral damage		Dislocation level	
		F statistic	***P value***	***F statistic***	***P value***	***F statistic***	***P value***	***F statistic***	***P value***
Bird type	1	0.16	0.690	5.98	<u>0.014</u>	5.51	<u>0.019</u>	0.03	0.874
Bird age	1	0.03	0.866	6.39	<u>0.011</u>	0.609	0.406	0.02	0.887
Bird weight	1	1.14	0.289	0.74	0.390	0.01	0.996	0.07	0.789
N1*		3.31	0.074	4.00	<u>0.050</u>	0.01	0.912	1.12	0.293
Killing method. Bird type	3	0.01	0.929	0.46	0.498	0	0.957	0.00	1.000
Killing method. Bird age	3	1.47	0.226	0.49	0.484	0	0.964	0.00	1.000
Killing method. Bird weight	3	0.5	0.655	0.79	0.394	0	0.957	0.00	1.000

* *Refer to Figure 3.1.*

4.3.2.2 Brain trauma methods: MARM and MZIN

For successfully killed birds (MARM = 15/40 birds; and MZIN = 6/40 birds), the percentage of birds for which the relevant head trauma post mortem factor was present, according to killing method is shown in Table 4.7. Killing method had no effect on the majority of post-mortem measures, apart from damage to left forebrain, mid brain, and brain stem. The MZIN was significantly more likely to cause trauma to the left forebrain and the mid brain compared to the MARM, however, the opposite was seen for the brain stem, with very few birds receiving the MZIN method sustaining damage compared to the MARM.

Table 4.7 Percentage of birds killed successfully for which the relevant head trauma post mortem factor was present, according to killing method, including GLMM analysis of killing method comparison. Significant *P* values are underlined.

	Percentage of birds			
Post mortem measure	**MZIN**	**MARM**	***F statistic***	***P value***
Skin broken	100.0	100.0	0.03	0.993
External bleeding	96.7	88.0	1.44	0.264
Subcutaneous hematoma	100.0	92.0	1.44	0.234
Skull damage	100.0	100.0	0.06	0.982
Left forebrain damage	62.5	0.0	5.81	<u>0.029</u>
Right forebrain damage	65.6	0.0	4.70	0.994
Cerebellum damage	65.6	64.0	0.00	0.998
Midbrain damage	84.4	0.0	5.80	<u>0.013</u>
Brain stem damage	31.3	92.0	5.10	<u>0.034</u>

No other factor or interaction had an effect on external bleeding, skin tearing, subcutaneous hematoma, and whether or not the skull was damaged. For each of the brain regions, the GLMM models are reported in Table 4.8. Bird type, bird age, bird weight and their interactions with killing method had no effect on damage to any region of the brain.

Table 4.8 GLMM output for brain damage regions. Significant *P* values are underlined.

Fixed effects	**df**	**Left forebrain**		**Right forebrain**		**Cerebellum**		**Midbrain**		**Brain stem**	
		F statistic	***P value***	***F statistic***	***P value***	***F statistic***	***P value***	***F statistic***	***P value***	***F statistic***	***P value***
Bird type	1	0.00	0.997	0.00	0.997	0.24	0.622	0.18	0.882	0.34	0.560
Bird age	1	0.00	0.971	0.00	0.971	1.77	0.186	1.52	0.341	1.65	0.201
Bird weight	1	0.05	0.480	0.51	0.480	1.09	0.299	0.58	0.671	0.20	0.654
Killing method. Bird type	3	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
Killing method. Bird age	3	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
Killing method. Bird weight	3	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00

4.4 Discussion

Evaluation of the killing potential of untried novel mechanical methods on cadavers was the first stage in the development of the devices. All four devices had been designed and prototyped with the aim to cause rapid loss of consciousness and brain death in order to be effective and humane. Ethically it would have been inappropriate to evaluated un-tested killing methods on live birds; therefore the aim of this study was to assess the physiological damage produced in cadavers as a result of each method, to infer killing potential.

The NMCD device was shown to have the highest killing potential (100%) compared to all other devices. However, all devices achieved a killing potential of over 60%. NMCD was also shown to have the highest device success (90%), demonstrating its consistency in achieving optimal damage to the cadavers, irrespective of bird type. Device success was always lower than the killing potential for each method. For the NMCD, MZIN and MARM the difference between the two was approximately 10%, demonstrating that 10% of the time each of these methods were not performing optimally (refer to Table 4.2). For NMCD, the primary reason for this difference was the number of carotid arteries severed, as on occasion only one was severed, as well as some birds receiving a lower dislocation level. In the case of MZIN, the few failures in device success were due to only region of the brain being damage or minor damage to all regions (e.g. internal brain cavity bleeding and bruising). Failures in device success with the MARM were primarily due to the spike not penetrating deep enough to cause complete severing of the brain stem, as well as slight issues with aiming, and the spike not penetrating the brain stem at all, but instead the cerebellum. In terms of brain trauma, this could reduce the chance of neurogenic shock and elongate the time to loss of consciousness and brain death (Alexander, 1995; Dumont et al., 2001a; Freeman and Wright, 1953; Kushner, 1998; White and Krause, 1993), but it did not appear to affect the inferred kill potential (i.e. the damage would still be fatal).

The MARM and MPLI had the joint lowest kill potential of 62.5%, however the MPLI had a significantly lower device success (27.5%) than its killing potential, as well as in comparison with other killing methods. The primary reasons being 55% of birds showed vertebral damage, failure of dislocation (55%) and 52.5% of birds showed trachea damage, which was representative of crushing injury and inference of causing death by asphyxiation, which is a serious welfare concern (Erasmus et al., 2010a; Gregory and Wotton, 1990; Salim et al., 2006; Sharma et al., 2005).

Bird age affected both killing potential and device success, in both cases revealing that it was easier to cause physiological trauma to younger birds and therefore easier to achieve the optimal level to achieve a reliable kill. Young birds are less physiologically mature, and therefore bones and cartilage are less calcified and re-enforced, as well as connective tissue being less fibrous, making dislocation and damage to the skull easier to achieve (Comi et al., 2009; McLeod et al., 1964; Sharma et al., 2005; Whittow, 2000; Williams et al., 1990). However, in terms of neck muscle and arterial tissue, aging can have a detrimental effect, with reduced elasticity in arterial walls and skeletal muscle, reducing stretching potential, therefore carotid arteries and neck muscle are more likely to tear when under strain (Benetos et al., 1993; Nair, 2005). However this needs to be considered in context of the size of the birds; smaller birds have less stretch potential than larger birds, therefore despite the increased elasticity, the magnitude of the stretch required to dislocate and tear counteracts this effect.

Post-mortem measures for neck trauma methods highlighted that the MPLI was more likely to cause skin tears and external bleeding, though not significant, which could be considered a practical issue in a commercial environment due to biosecurity, human health and safety as well as being visually un-appealing (Galvin, 2005; Gerritzen and Raj, 2009; Halvorson and Hueston, 2006; Kingsten et al., 2005; Nerlich et al., 2009). Worryingly, the MPLI, which was designed to dislocate the cervical vertebrae, only caused a dislocation 45% of the time and showed crushing injury to the trachea as well as the oesophagus. The injuries sustained, as well as the pressure applied by the blades, could still be fatal, but not necessarily by causing death by cerebral ischemia, which is the desired way (and considered the most humane) (Bader et al., 2014; Harrop et al., 2001; Taneichi et al., 2005; Veras et al., 2000). The primary concern with MPLI was that, despite the modifications, it was not performing in the intended way, indicating that it was not a reliable method and thus had limited killing potential.

Post-mortem measures demonstrated that both the MARM and MZIN always caused penetration of the skin and damage to the skull and the majority of birds bled into the external environment, as well as subcutaneously. There were significant differences in the areas of the brain that the devices damaged; however, this was not an issue, as they were designed to behave differently. With the MZIN, more than 60% of all birds received damage to the main areas of brain, excluding the brain stem, demonstrating diffuse damage across the brain, which the device is designed to do in order to cause concussion and brain death (Alexander, 1995; Finnie et al., 2000; Kushner, 1998; Oppenheimer, 1968). The MZIN showed higher killing potential than the unmodified Rabbit Zinger™, which had previously been reported to have a kill success rate of 50% in poultry (DEFRA, 2014). The MARM caused focalised damage to the brain stem and cerebellum, highlighting that the modifications to the MARM had adequately adapted its design to fit poultry. Damage to the brain stem theoretically would result in fatal functional impairment (e.g. puntilla) (Dembo, 1894; HMSO, 1995; HSA, 2004; Limon et al., 2009; Limon et al., 2010; Morzel et al., 2002; Widjicks, 1995). The un-modified Armadillo® was tested on poultry as part of a DEFRA report (2014), which reported it to have a low kill success of 46%, therefore the higher kill potential could be attributed to the modifications or that the killing potential was tested on cadavers, which are easier to handle, improving application of the method. The increase in success in the MZIN could be attributed to the same reasons.

Other factors were shown to impact some post-mortem measures (e.g. dislocation level, vertebral damage), demonstrating inconsistency dependent on the target species, although the impact was more associated with cervical dislocation methods than the head trauma methods. In general, broilers and younger birds were easier to cervically dislocate, although they are confounded, as by definition broilers at both ages tested were young immature birds. The result was also supported by the diameter of the neck also affecting dislocation potential, with smaller necks (younger birds) being easier to dislocate than larger necks. When considering vertebral damage, layers were more likely to receive damage, but again bird type was confounded with age, with laying hens being much older than any other bird group. The increased likelihood of vertebral damage could be attributed to the brittle bones of the laying hens (Whitehead and Fleming, 2000).

All other external factors had no impact on the post-mortem measures associated with brain trauma methods, indicating that these methods are less susceptible to inconsistency as a result of various types, size and age of birds. However, this has to be taken within the context that both of the brain trauma methods: MZIN and MARM had killing potentials of 84.2% and 62.5% respectively, both which suggest some issue with reliability.

This first study was a general assessment of the prototyped devices to ascertain if they showed killing potential. Three of the mechanical methods: NMCD, MARM and MZIN demonstrated killing potential, as well as consistency in physiological effects, with device success rates of over 50%, which also demonstrated that more than half the time the devices performed optimally. It was noted that in future studies more detailed assessment of post-mortem evaluations would be desirable, for example, damage location to the skull and size of dislocation (i.e. measurement of gap between two dislocated vertebrae), in order to establish in greater detail the effects on the birds’ anatomy and therefore more accurately infer the effect this may have on time to unconsciousness and brain death in live birds. The MPLI did not show consistency, and had a much lower device success of 27.5%, despite matching killing potential with the MARM. The abundant evidence of crushing injury in birds, was also a major concern, especially as the new European legislation on the Protection of Animals at the Time of Killing bans the use of any method which demonstrates death by crushing to the neck (European Council, 2009). As a result the MPLI were not taken forward in the project and following studies, based on an assessment that it’s potential and performance was not good enough to justify testing it on live birds.

5 Evaluation of electroencephalogram and reflex responses of anesthetised chickens killed using three mechanical devices

5.1 Introduction

Determination of loss of consciousness is fundamental to ascertaining the welfare impact of a killing method. Evaluation of brain function is a crucial element of defining the conscious state of the animal, as well as determining whether brain death (see Chapter 1.2) (Buchner and Schuchardt, 1990; Facco et al., 2002; Misis et al., 2008; Widjicks, 1995) has occurred and the killing method has been successful. However, defining and identifying the various states of consciousness and unconsciousness is problematic. Objective measures (e.g. electroencephalography (EEG), and presence/absence of reflexes) that indirectly infer consciousness states (including brain death) are currently the only way to establish vigilance states in livestock species (Anil et al., 1998; Blackman et al., 1986; Blackmore et al., 1995; Tidswell et al., 1987), including poultry (Gerritzen et al., 2004; Gregory and Wotton, 1990; McKeegan et al., 2013b; Sandercock et al., 2014). These measures are also used during anaesthesia in order to monitor anaesthetic depth and maintain an optimal surgical plane (Alkire et al., 2008; Nicolaou et al., 2012).

EEG data is one of the most useful tools in assessing the humaneness of on-farm killing as well as slaughter methods for all livestock species (Anil et al., 1998; Beyssen et al., 2004; Gibson et al., 2009; Tidswell et al., 1987). The EEG represents the electrical activity (i.e. potentials) of brain cells in the cerebral cortex via electrodes either surgically implanted on to the surface of the dura (technically an electrocortigram) or by resting electrodes on the scalp of the animal (technically an electroencephalogram) (Knudsen, 2005; McIlhone et al., 2014; McKeegan et al., 2013a; McKeegan et al., 2013b). The cerebral cortex is considered to be the primary region for generating consciousness in mammals (Baars et al., 2003), therefore the measuring of electrical potentials in this area provides information which can be related to consciousness and the frequency of electrical potentials is associated with changes in cortical metabolism (i.e. from reduction in oxygen availability and blood flow) (Boveroux et al., 2008; Velarde et al., 2002). For example, EEG recordings have been used to differentiate between varying states of sleep, unconsciousness and brain death (electrocerebral inactivity – ECI) (Baars et al., 2003; Johnson and Taylor, 1998; Raj and O'Callaghan, 2004; Sandercock et al., 2014; Velarde et al., 2002). EEG waveform analysis has also been tentatively used to infer subjective states (e.g. pain) in response to appropriate stimuli (e.g. noxious) (Johnson et al., 2005b; Murrell and Johnson, 2006).

In the field, it is not practical to record EEG in each animal to confirm unconsciousness and death (Erasmus et al., 2010c), therefore the presence/absence of reflexes are used to determine brain death (e.g. pupillary reflex, nictitating membrane reflex) (Anil, 1991; Anil et al., 1999; Blackmore and Delany, 1988; Coenen et al., 2009; Coles, 1997; Croft, 1961; Heard, 2000; Lawton, 1996) and loss of consciousness (e.g. jaw tone) (Erasmus et al., 2010c; Heard, 2000; Sandercock et al., 2014). The correlation between the loss of certain reflexes and EEG is not well documented in poultry (Gerritzen et al., 2004; Raj and Gregory, 1990; Raj et al., 1990; Sandercock et al., 2014), or other livestock species (Anil, 1991; Gregory and Shaw, 2000; Newhook and Blackmore, 1982; Shaw, 1989). However, a recent study demonstrated that the loss of jaw tone was indicative of an unconscious state in layer hens and turkeys, when the state was induced through anaesthesia by sevoflurane (Sandercock et al., 2014). That study also showed that the loss of the nictitating membrane reflex was a conservative indicator of death in layer hens and turkeys (Sandercock et al., 2014). It also clearly demonstrated the validity of EEG power analysis in differentiating between varying states of consciousness, unconsciousness and brain death (Sandercock et al., 2014). The results showed a clear change in the EEG signal pattern in behaviourally confirmed unconscious states, with unconsciousness accompanied by a sharp increase in total spectral power (PTOT), which is associated with a decrease in the median frequency (F50) and the spectral edge frequency (F95) (Sandercock et al., 2014).

The aim of this experiment was to evaluate the humaneness of three killing treatments (MARM; MZIN; and NMCD) and a control treatment (MCD) for on-farm killing of poultry. The three killing treatments had been developed and trialled in cadavers before this experiment (see Chapter 4), and were shown to produce sufficient physiological trauma in order to result in death, as well as performing in the designed way. However, because of the uncertainty of the humanness of any new devices, anesthetised chickens were used. In this trial the efficacy of killing was assessed by EEG (i.e. brain activity) analysis, ECG (i.e. heart rate) analysis; behavioural/reflex measures. Post-mortem analysis of the physiological damage produced in anaesthetised broilers and layers was also carried out.

5.2 Materials and Methods

5.2.1 Animal housing and husbandry

The experiments were conducted between September 2012 and February 2013. A total of 232 female layer-type and meat-type chickens were used for the study across four batches and distributed across two types and ages (Table 5.1). Birds were collected from commercial farms and transported to SRUC facilities in four batches; 40 birds in batch one and 64 birds per batch for batches two, three and four. Each batch contained the four bird type x age combinations. All birds were weighed and wing-tagged on arrival. The birds were housed for two weeks prior to the experiment in order to allow them to acclimatise to the new environment, as well as for specific birds to undergo EEG electrode implantation surgery and post-surgical recovery. Birds were housed in separate rooms per bird type and age group to provide recommended environmental controls (Aviagen, 2009; Hy-Line, 2012). All birds were kept in floor pens with wood-shavings litter and kept at lower than commercial stocking density and with suitable environmental enrichments (DEFRA, 2002a; DEFRA, 2002b) (Table 5.1). All pens were constructed from a wooden frame with wire-grid sides and roofs, allowing visual and auditory contact with other birds within the same room. Broiler chicks, which were not implanted with EEG electrodes due to their small size, were housed in one pen as a group (L 1.5 m x W 2.5 m x H 1.5 m). Broilers (slaughter-age), layer pullets and layer hens were kept in pairs prior to EEG implantation surgery and singly (with visual and auditory contact with others) post-surgery. Pen sizes were L 1.5 m x W 0.5 m x H 1.5 m. All birds had *ad libitum* access to appropriate food and water. All birds were inspected twice daily, and the minimum and maximum temperatures were recorded each morning.

Table 5.1 Accommodation and bird details for each bird type and age group.

Bird group	N	Mean bird age on arrival (days)	N per pen	Pen furniture
Layer pullets (Lohmann strain)	64	63.7 ± 0.3	1-2	1 feeder, 2 automatic cup drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string
Layer hens (Lohmann strain)	64	485.1 ± 0.5	1-2	1 feeder, 2 automatic cup drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string
Broiler chicks (Ross 308 strain)	40	13.3 ± 0.2	10	2 x feeder, 1 x automatic large bell drinker, 4 x suspended shiny objects
Broiler (slaughter age) (Ross 308 strain)	64	32.3 ± 0.2	1-2	1 feeder, 2 automatic cup drinkers, 2 x suspended shiny objects

5.2.2 Study Design

Three mechanical poultry killing devices, MARM, MZIN and NMCD were assessed for their kill efficacy alongside the control method - MCD. The device designs are described in detail in Chapter 3.3. All of the mechanical devices were tested in a previous experiment on cadavers and had demonstrated their ability to kill birds (Chapter 4.3). MCD was performed following the HSA's guidelines with the method described in Chapter 3.4.

The four killing treatments were tested on 232 unconscious birds across two bird types and ages. The original experimental design involved 160 birds (10 birds per bird type and age for each killing treatment), however following the completion of batch one it was identified that for two of the killing treatments (NMCD and MCD), the presence of the EEG electrode on the bird's head may have an impact on the kill efficacy, therefore additional birds not implanted with EEG electrodes were added to these two killing treatments in order to incorporate an electrode implant effect into the analysis. Therefore for NMCD and MCD 22 birds per bird type and age were killed, except for broiler chicks which were not implanted (Table 5.2). For the MARM and MZIN, 10 birds per bird type and age were killed. The presence of the EEG electrode was also shown to cause an issue with the MZIN treatment, following completion of batch one, with the bolt repeatedly dislodging the implant on impact, rendering data recording impossible. As a result, remaining birds for the MZIN treatment did not undergo implantation surgery.

Table 5.2 Numbers of birds allocated to the four killing treatments.

Bird type	Bird age	EEG electrode Implanted	MARM	MZIN	NMCD	MCD
Layer	Pullet	Y	10	3	10	10
		N	-	7	12	12
	Hen	Y	10	2	10	10
		N	-	8	12	12
Broiler	Chick	Y	-	-	-	-
		N	10	10	10	10
	Slaughter age	Y	10	2	10	10
		N	-	8	12	12
N total per device			40	40	76	76

Across the four batches, a Graeco Latin-Square design was used to systematically randomise killing treatment, bird type and age and kill order for the original 160 birds. Killing treatment was allocated to individual birds so as not to confound killing treatment with pens. Birds were killed over four days for each batch, with 10 birds killed per day. The additional non-implanted birds for MCD and NMCD (12 birds per bird type and age per batch) were incorporated without interfering with the original design by adding a second session on each day in the order specified by the original Graeco Latin-Square design. A one hour rest period between the kill sessions within each day was implemented prevent operator muscle fatigue affecting the results.

Elastic bandage (Vetrap^{TM}) was wrapped around the bird's body and over the wings immediately prior to killing and prior to anaesthetic induction in order to minimise excessive wing movement related to tonic and clonic convulsions, which could hamper the visibility of recorded behavioural measures as well as create artefacts in the EEG and ECG traces which make them unusable (Abeyesinghe et al., 2007; Becker et al., 2010; McKeegan et al., 2013b; Sandercock et al., 2014).

All birds were anaesthetised immediately prior to the killing treatment being applied via a "fast knockdown" method using a face mask (induction via gas inhalation of 7.4% sevoflurane (SevoFlo, Animal Health, Hampshire, UK) and 92.6% oxygen, at 2 litres/min for approximately 20 s). This was done to protect bird welfare, as the devices had only been previously tested on cadavers. All killing treatments were applied by one trained and experienced operator (JM). The efficacy of each device was determined in four ways: (1) analysis of cardiac activity (via electrocardiogram (ECG) recordings); (2) analysis of electrical brain activity (via EEG recordings); (3) duration of reflexes and behaviours post killing treatment application; and (4) post mortem evaluation.

The experiments were digitally video recorded by two cameras (Low-lux B/W waterproof cameras: SK-2020XC/SO, RF Concepts Ltd, Belfast, Ireland and Geovision GV-DVR, ezCCTV Ltd, Herts, UK) from the point of killing treatment application through to 30 s after all behaviours and reflexes had ceased. The video footage from both cameras (camera 1 was aimed at the bird's body; camera 2 was aimed and zoomed in on the bird's head) allowed back-up observations to be performed, if live observations of behaviours and reflexes were missed.

5.2.2.1 Kill success

Kill success was defined as only one application attempt with no signs of recovery (recovery was indicated by sustained and/or return of rhythmic breathing and reflexes such as jaw tone). If any signs of recovery continued for 15 s (i.e. 1 interval measure) the bird was immediately emergency euthanised; the method of euthanasia was killing treatment-dependent in order to prevent post mortem examination data being voided (e.g. for MCD and NMCD it was the CPK 200 (Accles and Shelvoke, 2010); for the MARM and MZIN it was MCD). The kill was recorded as a failure, and reflex/behaviour duration data was no longer recorded. Emergency euthanasia by sodium pentobarbital injection (Euthatal, Merial Animal Health Ltd., Essex, UK) (AVMA, 2007; Sandercock et al., 2014) was not practical for application since the bird's wings were bound by the elastic bandage, which would result in a delay in administration in order to gain access to a brachial vein, which would compromise the bird's welfare further.

Device success was defined as the killing treatments producing the optimal trauma to the bird, specific to the killing treatment's design. For example, for the MARM this was penetration through the foramen magnum of the skull and severing of the brain stem (Limon et al., 2009; Limon et al., 2010). For the MZIN penetration of the skull and severe brain damage to a minimum of one area of the brain was expected. For the control and NMCD, device success was defined as full dislocation of the neck at C0-C1, severing of the spinal cord and both carotid arteries and no tears or breaks to the skin (as recommended by HSA (2004)).

5.2.2.2 ECG Recordings

All birds had two ECG surface (non-invasive) recording electrodes (Blue Sensor, Ambu™ Ltd., Henry Schein Medical, London, UK) attached to cleaned, feather-free skin under the wings (above the *pectoralis* muscles on either side of the sternum), with the use of tissue adhesive (Vetbond™) . The electrodes were attached prior to killing treatment application and were further secured by the elastic bandage secured over the wings. The electrodes were connected to a battery-powered telemetry logging device, which also recorded the EEG activity (Lowe et al., 2007; McKeegan et al., 2011; Sandercock et al., 2014). The loggers contained industry-standard micro-SD memory cards (SanDisk 32GB, Maplin Electronics Ltd. Rotherham, UK) for storing data. Continuous sampling of ECG activity was logged at 1 kHz (Lowe et al., 2007) for a minimum of two minutes prior to killing (baseline recordings), until the bird's death and all behavioural data had been recorded, after which the logged data was downloaded. On a rotational basis three identical loggers were used in order to minimise the risk of a logger failure. The loggers were housed in an adjustable Lycra™ body harness on the bird's back.

5.2.2.3 EEG Recordings

EEG Electrode construction

The electrodes used to detect EEG activity were custom built and used only once. The construction method has been described and validated previously (McKeegan et al., 2013b; McKeegan et al., 2011; Sandercock et al., 2014). The three pin DIN (Deutsches Institut für

Normung) socket (RS Components Ltd, Corby, UK) acted as the base and connector for each electrode. The three pin DIN connection loops located on the ventral side were removed and three lengths of Teflon coated silver wire (0.35 mm) (World Precision Instruments Ltd, Hertfordshire, UK) were soldered to the sites (wire lengths: 2 x 1.5 cm; 1 x 2.0 cm) (Figure 5.1). The soldered wire ends had the Teflon coat removed in order to expose the silver wire to the connector site. The connector sites and base of the DIN plug were insulated from electrical noise and protected by a layer of dental cement (Duralay, Dental Directory Ltd, Witham, UK), which formed a smooth flat cap.

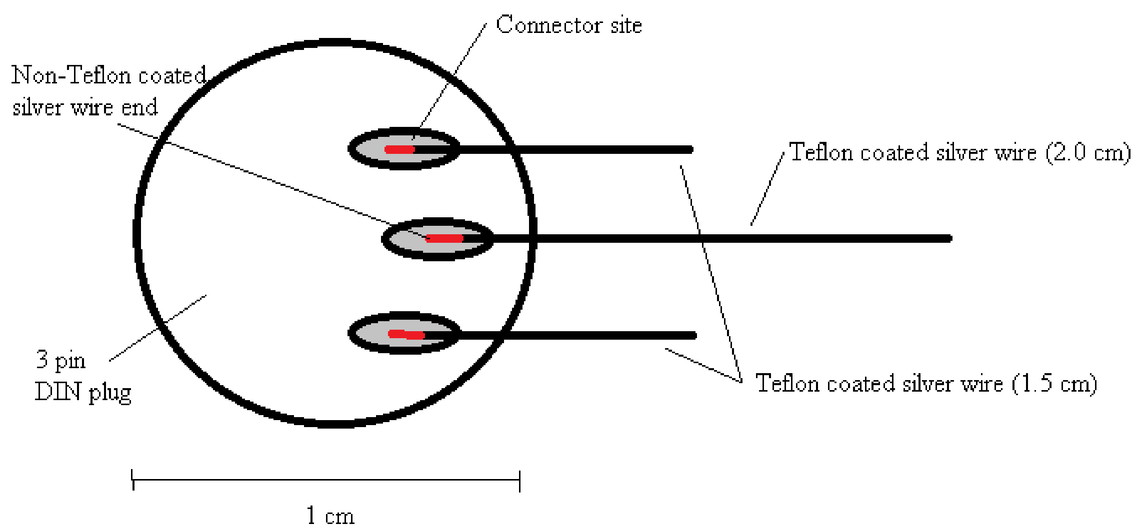


Figure 5.1 Three pin DIN socket and locations of silver wire connections. The longest middle wire acts as the reference electrode and the two shorter wires either side act as the bipole electrodes.

EEG electrode surgical implantation

Due to body size and physiological maturity, only layer pullets, laying hens and slaughter-age broilers were implanted with EEG electrodes and had EEG data collected during killing. The EEG electrode measured 14.0 mm in diameter, while a 2-3 week old broiler chick's head had a mean diameter of 22.5 mm (refer to Chapter 3.1), therefore it was considered too small to support the electrode and the skull too soft (McLeod et al., 1964) to cope with the implantation. A total of 97 birds were implanted across the four batches.

The implantation surgery process was performed on a single bird at a time. An EEG electrode was surgically implanted, under general anaesthesia, a maximum of six days prior to killing. The bird underwent feed withdrawal for a maximum of four hours prior to surgery. The bird was transported in an animal carrier cages from its home pen to the surgical suite and recovery area, the bird was weighed and then housed individually within the recovery area in a holding cage (L 0.9 x W 0.6 x H 0.7 m), which was covered with fleece blankets in order to maintain temperature, as well as to create a dark interior to minimise disturbance and stress. Birds as well as other species are susceptible to hypothermia during and post-surgery (Beilin et al., 1998; Buggy and Crossley, 2000), therefore the temperature of the recovery area and surgical suite was monitored hourly and maintained at 22–23 ºC. The bird was injected intramuscularly into the pectoral muscle with a pre-medication (dexmedetomidine; Dexdomitor, Elanco, Animal Health, Hampshire, UK) approximately 30 minutes prior to surgery. The dosage was 80 mg/kg for all birds. Following injection the bird was returned to its holding pen to allow sedation to take place.

Once the bird showed clear signs of sedation (i.e. drooping of wings, sitting, eyes closed), it was removed from its holding pen and taken to the surgical suite, where anaesthesia was induced via a face mask and gas inhalation of sevoflurane at a concentration of 8% vaporised in 100% oxygen as described above. Once the bird was unconscious, evaluated by lack of response to a sharp toe pinch, intubation of the trachea was performed with a PVC uncuffed endotracheal tube (Smiths-Medical, Ashford, UK) (4 mm tube for slaughter-age broilers and laying hens and 3.5 mm tube for layer pullets). General anaesthesia was maintained with sevoflurane concentration ranging between 1.5 – 4.0% vaporised in 100% oxygen (Table 5.3), until the EEG electrode implantation had been completed, which usually took approximately 15 minutes. Prior to the commencement of the surgery, carprofen (a non-steroidal anti-inflammatory drug; Rimadyl, Zoestis UK Ltd, London, UK) was injected sub-cutaneously at the nape of the neck, at a dosage of 4 mg/kg to provide post-operative pain relief. Several physiological variables (heart rate, respiration rate, blood pressure, end-tidal CO_2, and sevoflurane) were recorded and monitored during surgery using a multi-parameter monitor (Mindray Beneview T5, Mindray Medical International, Nanshan, China).

Table 5.3 Details of general anaesthesia (GA) induction and maintenance for each bird type

Bird type	GA Induction			GA maintenance		
	Sevoflurane concentration (%)	**Oxygen concentration (%)**	**Oxygen flow rate (L min^{-1})**	**Sevoflurane concentration (%)**	**Oxygen concentration (%)**	**Oxygen flow rate (L min^{-1})**
Layer pullet	8	100	4.0	2.0 – 4.0	100	1.5 – 2.0
Laying hen	8	100	4.0	1.5 – 3.5	100	1.5 – 2.0
Broiler (slaughter-age)	8	100	4.0	1.0 – 2.5	100	1.0 – 2.0

The EEG electrode implantation procedure has been previously described (McKeegan et al., 2013b; McKeegan et al., 2011; Sandercock et al., 2014). The bird's head was secured in with blunt ear bars in order to restrict movement. Feathers from the top of the head and behind the comb were removed (circular area of approximately 2.5 cm in diameter) and the skin was cleaned with Ethanol (Ethanol – 100%, Henry Schein Medical, London, UK). Two incisions approximately 1 cm in length were made in order to create to a cross-shaped incision in the skin behind the comb. The four flaps of skin created by the cross-shape were then secured and draped with haemostats either side of the ear bars (left and right, anterior and posterior). Two holes (2.0 mm in diameter and depth) were drilled into the occipital bone of the skull, approximately 1 cm apart and two nylon (cheese-head) screws (M4 - RS Components Ltd, Corby, UK) were inserted. The DIN socket was secured with dental cement between these two screws and anchored to the skull. Two further holes were drilled through either side of the sagittal suture of the cranium over the left and right telencephalon, exposing the dura. The bipolar electrode wires were trimmed to length and inserted through the drill holes, in order to contact the dura. The reference electrode was inserted under the skin between the comb and the skull. The electrode wires were covered and insulated with dental cement, securing them in position and reinforcing the attachment to the skull. The four skin flaps were then sutured together (Prolene Blue, Ethicon, Johnson and Johnson Medical Ltd, Livingstone, UK) in order to close the wound around the EEG electrode implant. Once the suturing was completed the vaporiser was turned off and oxygen flow was increased to 2-3 L min^{-1}. Once the bird showed signs of recovery (e.g. cough reflex), it was extubated and the face mask was placed over its head to provide oxygen and aid recovery. When the bird regained muscle tone it was returned to the individual holding pen in the recovery area and was monitored regularly. After the bird was standing and showing no effects of the anaesthetic (approximately 15-20 minutes) it was returned to the home pen area and housed in an individual pen and provided immediately with food and water.

EEG recording and processing

The EEG and ECG recordings were simultaneously logged via the telemetry logging device, secured to the bird's back in a Lycra harness (see Section 5.2.2.2) (Lowe et al., 2007; McKeegan et al., 2007; McKeegan et al., 2011). EEG activity was recorded at 1 kHz (1000 sample points per second) and was sampled continuously during a resting two minute baseline period (an awake bird held by a technician), during "fast knockdown" of anaesthetic, during killing and post-kill activity until all behaviours and reflexes had ceased for a minimum of 30 s. The logged data were immediately transferred from the micro-SD memory card to a laptop PC and an external hard-drive in order to create two back-up copies of the data files. Excerpts of EEG activity were then analysed based on 2 s epochs which were visually identified as artefact free from the raw traces. These underwent spectral power analysis using a Fast Fourier Transform (FFT) Algorithm (1024 Hanning window - Spike2, v4.2, Cambridge Electronic Design, Cambridge, UK) (McKeegan et al., 2013b; Sandercock et al., 2014). This analysis treats the EEG waves as a series of weighted sinusoids enabling data in the time domain to be converted to data in the frequency domain.

Three excerpts (midpoint ± 10 s either side) of EEG wave activity were obtained and analysed in the 2-min baseline period (conscious bird), in order to generate mean parameters for individual awake birds. One excerpt was taken during the "fast knockdown" period, when the bird was confirmed as unconscious due to unresponsiveness to painful stimuli (e.g. 20 s after the start of anaesthetic induction). Overlapping 2 s epochs were obtained from -2 s to +5 s (i.e. -2 to 0, -1 to +1, 0 to +2, +1 to +3, +2 to +4, and +3 to +5 s) relative to the time of killing treatment application (estimated kill time = 0 s). From +5 s to +59 s, a continuous series of non-overlapping 2 s epochs were analysed. Thereafter 2 s epochs were sampled from the midpoint every 15 s, until three consecutive samples were judged to be isoelectric.

5.2.2.4 Behavioural Observations

Five cranial reflexes and behaviours (jaw tone, pupillary, nictitating membrane, rhythmic breathing, and cloacal movement) and two death-related behaviours (clonic wing flapping and leg paddling) (Table 5.4) were assessed as present or absent in 15 s intervals post killing treatment application, until a consecutive 30 s absence of all behaviours and reflexes was observed. All of these reflexes and behaviours have been validated in previous research as indicators of either brain death or unconsciousness (Anil et al., 1998; Erasmus et al., 2010a; Erasmus et al., 2010c; Sandercock et al., 2014). The 15 s interval was assigned following pilot work which indicated that this was appropriate length of time to accurately assess all reflexes and behaviours before having to begin the next observation interval. Assessment of the presence and absence of the behaviours and reflexes was conducted by two observers: observer 1 assessed reflexes and behaviours associated with the bird's head, while observer 2 assessed measures relating to the body and limbs of the bird. Head and body measures were recorded simultaneously by both observers, but in a specific order within each observer (i.e. head measures were always measured in the order of jaw tone, nictitating membrane and pupillary reflex; while body measures were recorded in the order of rhythmic breathing, wing flapping, leg paddling and cloacal movement). One-zero sampling methods were used, meaning that if a reflex/behaviour was present during any point of a 15 s interval it was defined as present for the entire interval (Martin and Bateson, 2007), providing a maximal measure of reflex/behaviour durations post killing treatment to therefore infer a conservative measure of consciousness. Data are reported as the mean of the maximum durations. If a reflex or behaviour could not be recorded (e.g. pupillary reflex was concealed due to damage to the eye) the data was recorded as missing.

Table 5.4 List of reflexes (above dotted line) and behaviours (below dotted line) recorded post-killing treatment, with the specific cranial nerve pathway and identified brain area for control 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.

**(Erasmus et al., 2010c; Knudsen, 2005; Van de Sluis et al., 2009; Whittow, 2000)*

5.2.2.5 Post-mortem evaluations

A post-mortem examination was performed on every bird immediately after all behaviours and reflexes had ceased for a minimum of 30 s and the bird was confirmed to be dead. Specific post-mortem measures were recorded for each killing treatment as their target areas were different. For all killing treatments binary yes/no measures were recorded for skin broken, external blood loss and subcutaneous hematoma.

For the MZIN and MARM, seven specific measures were recorded: skull penetration location (see Figure 5.2 for classified skull regions); binary yes/no measures of damage to the left forebrain, right forebrain, cerebellum, midbrain and brainstem; and the presence of an internal brain cavity hematoma.

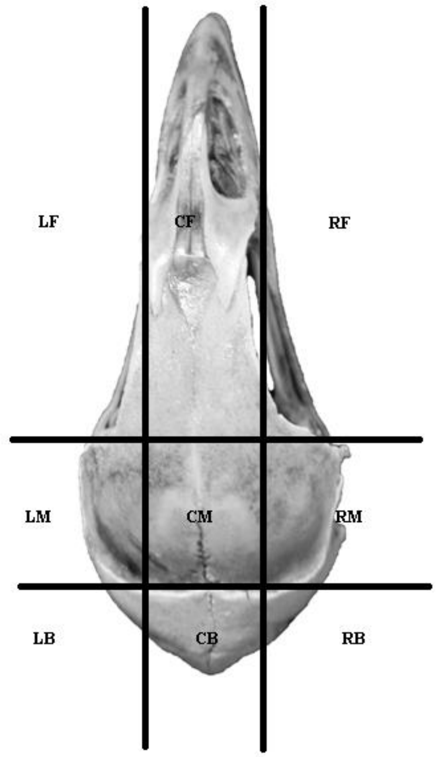


Figure 5.2 Photograph of a poultry skull indicating the nine skull penetration areas mapped: areas are separated into 3 regions: Front (F), Mid (M), and Back (B) and then split into the left (L), centre (C) and right (R) sides. Specimens prepared and photographed by author.

For killing treatments which caused trauma to the neck of the bird, seven specific post-mortem measures were assessed: four binary measures (yes/no) were taken for dislocation of the neck, vertebra damage (e.g. intra-vertebra dislocation/break), damage to neck muscle, and whether the spinal cord was severed. The level of cervical dislocation was recorded (e.g. between C0-C1, C1-C2, C2-C3, etc.), as well as a measurement of the length (cm) of gap between the dislocated cervical vertebra. The number of carotid arteries severed was also recorded as either zero, one or both.

5.2.3 Statistical Analysis

All data collected at the bird level were summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat (14th Edition). Statistical significance was termed by a threshold of 5% probability based on F tests. Summary graphs and statistics were produced at the bird level. For all models the random effects included the batch, date and the bird ID. All fixed effects were treated as factors and classed as categorical classifications.

5.2.3.1 Kill success

Statistical comparisons for kill success and device success were conducted via Generalised Linear Mixed Models (GLMMs), using logit link function and binomially distributed errors due to the nature of the binary data. In the maximal models, fixed effects included killing treatment, bird type, bird age, EEG implant, bird weight and all their interactions. Dispersion was fixed at one.

5.2.3.2 ECG data

The ECG waveform recordings were uploaded and viewed in Spike2 (Spike2, v4.2, Cambridge Electronic Design, Cambridge, UK); a data acquisition and analysis program (Abeyesinghe et al., 2007; Sandercock et al., 2014), which allowed raw ECG traces to be automatically converted into heart beats per minute.

5.2.3.3 EEG data

Following data processing (detailed in Section 5.2.2.3) of the EEG data in Spike2, novel coded programs were written for further processing and calculations of bird level summaries were conducted in Genstat (14^{th} Edition). For each 2 s epoch, FFT analysis was used to produce an EEG power spectrum from which three key spectral variables were calculated within the coded Genstat programs: Total power (PTOT); Median frequency (F50) - the frequency below which 50% of the EEG power resides, and spectral edge frequency (F95) - the frequency below which 95% of the EEG power resides (McKeegan et al., 2007; Tonner, 2006). Electrocerebral inactivity (ECI) (also termed isoelectric) is visually identifiable (Abeyesinghe et al., 2007; Coenen et al., 2009; McKeegan et al., 2013b; Sandercock et al., 2014), which allowed spectral variables to be attributed to the brain activity state (Table 5.5). Based on rapid knockdown data (summarised in Table 5.5), a state of unconsciousness was defined as an F50 less than 12.7 Hz and a PTOT higher than 850 mV.

Table 5.5 Descriptive statistics (mean, SE, maximum and minimum) of spectral variables (PTOT and F50) established for awake, unconsciousness (anaesthetised) and brain death states in broilers and layers.

Conscious state	Identifiable method	N	PTOT (mV)				F50 (Hz)			
			Mean	**±SE**	**Min.**	**Max**	**Mean**	**±SE**	**Min.**	**Max**
Awake	- behavioural observation	184	739.7	53.5	178.6	4590.7	23.9	0.7	17.8	48.6
Unconscious	- anaesthetised - no response to noxious stimuli	62	4287.5	455.4	850.9	16494.8	6.8	0.2	4.9	12.7
Brain death	- ECI visually identified	341	72.2	1.4	16.3	170.3	26.8	0.8	17.1	49.7

In order to prevent a large number of two second epoch samples being omitted due to noise artefact (“mains hum” 48.83 - 51.76 Hz noise peak) (Delorme and Makeig, 2004; Gwin et al., 2010; Lowe et al., 2007), a novel post-hoc data filtering method was created which involved fitting a regression line by linear interpolation. The linear regression was fitted to the FFT output versus the actual spectral frequency to ten data points; five points either side of the noise peak, and then replaced the data spike by points from the fitted regression line. Therefore the samples containing the noise peak are not removed, like in other filtering methods (e.g. notch filtering or band pass filtering (Delorme and Makeig, 2004)) but are kept within the data set, allowing the power spectrum to remain complete. Pilot work (n = 88 birds, 1166 epochs) demonstrated that the calculated spectral variables were highly correlated between filtered and non-filtered 2 s epochs (e.g. PTOT (r = 1.000, $P < 0.0001$); F50 (r = 1.000, $P < 0.0001$); F95 (r = 0.999, $P < 0.0001$)), demonstrating that the new method of filtering affected the power spectrum analysis less than the more crude filtering methods used in current programs (Delorme and Makeig, 2004; Gwin et al., 2010). For successful kills only, the durations to first and last time that F50 ≤ 12.7 Hz and ≤ 6.8 Hz, as well as the first and last time to the trace becoming isoelectric (PTOT < 170 mV; and F50 > 17 Hz) were modelled through GLMMs with logarithm function for Poisson distribution. Non-successful kills were emergency killed, and so excluded from EEG analysis.

The approach used represented a balance between ensuring that birds were unconscious when killed but minimising ongoing effects of anaesthesia on the EEG after killing. Light anaesthesia as induced by masking is transient, and birds readily recover (within approximately 4 s, unpublished observations), so if not killed the birds would have quickly regained consciousness. The mean time it took to remove the bird’s head from the mask, position it, and apply the killing treatment was 2.4 s, so there would have been some effects on EEG post kill. To allow for this, the epochs which were within the first 2 s post-killing were removed from the analysis for calculating time to unconsciousness (F50 $\leq$ 12.7 Hz and $\leq$ 6.8 Hz), in order to minimise the effects of the anaesthetic and increasing validity of the results.

Summary statistics and graphs were produced at the bird level, while statistical comparisons focussed on estimated means and differences between means. Fixed effects included in the maximal model were device, device success, bird type, bird age, bird weight, and their interactions. Further analysis involved sub-setting the data for NMCD and MCD treatments only (excluding MARM), which allowed post-mortem fixed effects (e.g. cervical dislocation point and carotid arteries severed) to be fitted into the models as factors. In the case of the modelling post-mortem effects for the MARM there was insufficient variation to allow analysis.

5.2.3.4 Behavioural and reflex data

For the reflex/behaviour durations, statistical comparisons were performed on a sub-set of data to remove kill failure birds, in order to prevent data skewing. The presence/absence of each reflex and behaviour was summarised into interval counts (e.g. present in 0-15 s = 1 count), therefore summarising the data into mean maximum interval counts at the bird level for each reflex, which were then converted back into the time dimension(s) for reporting descriptive statistics. GLMMs with logit link function and Poisson distributed errors were fitted to the interval counts. Overall statistical comparisons across the killing treatments were conducted. Fixed effects included in the maximal model were device success, bird type, bird age, bird weight, and the interactions between them. Further analysis involved sub-setting the data into two groups: (1) NMCD and MCD; and (2) MZIN and MARM, which allowed post-mortem fixed effects (e.g. (1) cervical dislocation point and carotid arteries severed; (2) binary measures of specific brain region damage) to be fitted into the models as factors.

5.2.3.5 Post mortem data

Data was subset twice, initially to remove unsuccessfully killed birds (i.e. kill success “no”) in order to prevent data skewing; and then into two groups: 1) NMCD and MCD (control); and (2) MZIN and MARM, in order to allow logical comparison between killing treatments which damaged the neck or the head. All post-mortem binary measures (e.g. skin break, subcutaneous hematoma, etc.) and categorised measures (e.g. cervical dislocation level, number of carotid arteries severed, etc.) were conducted via GLMMs using logit link function and binomial distribution. Fixed effects included were killing treatment, bird type, bird age, EEG implant, bird weight, and their interactions. For killing treatments which damaged the neck, some variables were also included as factors in modelling for other variables (e.g. variable = dislocation level, factor = neck gap size). For killing treatments which damaged the head, the variable of skull penetration location was also included as a factor in modelling for the binary variables of brain regions damaged (e.g. brain stem, cerebellum, etc.).

5.3 Results

Two birds died prior to the killing date, the first a slaughter-age broiler which was humanely euthanised upon arrival at the experimental site, due to leg health issues (intended killing treatment: NMCD) and the second was a layer pullet which died after EEG electrode implantation surgery due to post-operative complications (intended killing treatment = MARM). Therefore the N for these two killing treatments was reduced by one (MARM = 39 birds; NMCD = 75 birds). For the remaining birds, mean body weights at the time of killing were: layer pullets 0.88 ± 0.02 kg; layer hen 1.76 ± 0.26 kg; broiler chick 1.02 ± 0.04 kg; slaughter-age broiler 2.49 ± 0.06 kg. Mean bird ages at the time of killing were: layer pullet 11.3 ± 0.1 wks; layer hen 71.8 ± 0.3 wks; broiler chick 21.1 ± 1.0 days; and slaughter-age broiler 40.5 ± 3.0 days.

5.3.1 Kill Success

A total of 33 birds were not killed in the first attempt across the killing treatments: MARM = 19/39 birds; MZIN = 30/40 birds; NMCD = 72/75 birds; and MCD = 0/76 birds. These birds were immediately emergency-killed, invalidating their reflex/behaviour, EEG, ECG and post-mortem data. Anecdotally, both the MARM and MZIN were difficult to apply and required a short period of time to aim and position the birds correctly, despite the birds' being anaesthetised. For the MARM, the correct insertion cup had to be placed in position and adjusted if necessary (refer to Chapter 3 – Table 3.2). For the MZIN, the bolt muzzle had to be pushed down on the birds' heads with noticeable pressure in order to prevent re-coil, as well as maximise the chance of an accurate shot. For the three birds which were unsuccessful for the NMCD, a second immediate attempt was required. All three of these birds were slaughter age broilers with a mean weight of 3.57 ± 0.2 kg and in the 95th percentile of all bird weights tested here, irrespective of killing treatment.

Kill success ($F_{3,229}$ = 24.46, P < 0.001) was significantly affected by killing treatment. MCD was the most successful method, with 100.0% overall percentage kill success, followed by NMCD with 96.0%; MZIN with 75.0%; and MARM with 48.7%. Bird type, bird age, EEG implantation, bird weight, kill order and all interactions did not have a significant effect on kill success. Device success was significantly affected by killing treatment ($F_{3,229}$ = 4.38, P = 0.004), with the MZIN being the most successful (75.0 ± 0.0%) and matching its kill success. The NMCD, MCD and MARM all had less than 45% device success overall (Figure 5.3). Device success was also affected by bird age ($F_{6,229}$ = 4.48, P = 0.034), with device success being easier to achieve in younger birds compared to older birds. Both bird type ($F_{1,229}$ = 3.27, P = 0.070) and EEG implant ($F_{1,229}$ = 3.27, P = 0.070) had a tendency to affect device success, with layer type birds and EEG implanted birds less likely to achieve a device success. Bird weight, kill order and all interactions did not have a significant effect on device success.

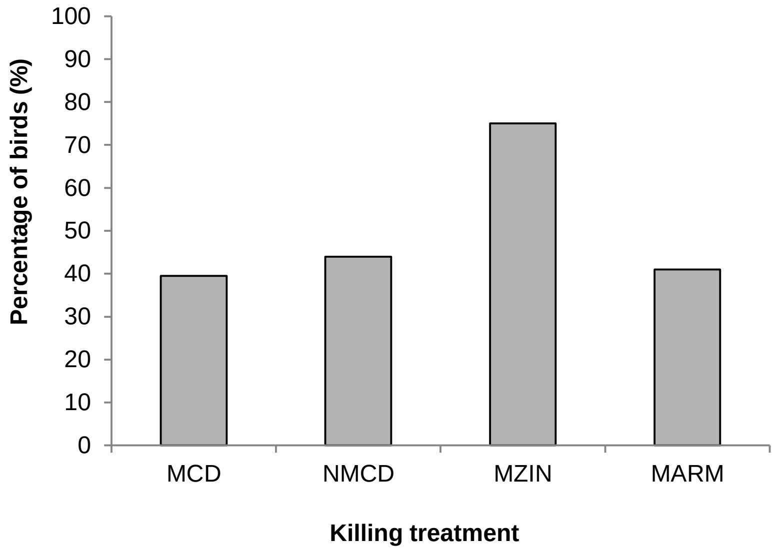


Figure 5.3 Summary of device success rates (%) across the four killing treatments.

5.3.2 ECG Recordings

The recording of the ECG data was not successful. The self-adhesive ECG electrodes (Blue Sensor, Ambu™ Ltd., Henry Schein Medical, London, UK), did not maintain contact with the skin in the first batch. For following batches, additional tissue adhesive (Vetbond™) was used, however the data recording was still impaired when birds started convulsing after killing. As a result, ECG data for 182 birds (79%) was available pre-treatment application, but only for 12 birds (5%) post-treatment application. However, these 12 birds also had extensive areas of missing data due to convulsive muscle activity interfering with the ECG recording. Therefore there was not enough ECG data to perform a meaningful analysis.

5.3.3 EEG Recordings

In total, 74 out of 95 birds (77.9%) that were implanted generated complete or partial EEG traces from baseline to knockdown, killing, post-kill and isoelectric. The remaining 21 birds were from batch 4, where no birds had EEG successfully recorded post-baseline due to a technical fault with the wire connection between the implant and loggers, which resulted in disruption to logging of clean EEG signal during even minor bird movements.

Out of the 74 birds with EEG successfully recorded, 58 birds were successfully killed, therefore a maximum of 58 traces with 2726 epochs were available for analysis. These 58 traces were unevenly spread across the three killing treatments and the three implanted bird types and ages (Table 5.6). Visual evaluation of these traces established that 1166 epochs were considered “clean” and artefact-free, however with the use of the designed novel filtering method, a further 512 epochs were eligible for data processing, totalling 1678/2726 epochs being available for analysis (61.6%). The remaining 38.4% were unusable for several reasons (e.g. loss of signal to recording device, significant movement artefact, etc.).

Table 5.6 Distribution of useable EEG traces across the three killing treatments and bird groups.

	Killing treatment		
Bird group	**MCD**	**MARM**	**NMCD**
Broiler (slaughter age)	8	5	7
Layer pullet	7	5	7
Layer hen	8	3	8

In the baseline period (awake/conscious) the spectral variables of PTOT ($F_{2,176}$ = 1.13, P = 0.290), F50 ($F_{2,176}$ = 1.28, P = 0.346) and F95 ($F_{2,176}$ = 1.19, P = 0.298) were not significantly different between killing treatments. There were also no significant differences between killing treatments for PTOT ($F_{2,48}$ = 0.35, P = 0.795), F50 ($F_{2,48}$ = 0.47, P = 0.982) and F95 ($F_{2,48}$ = 0.40, P = 0.833) for the knock-down (unconscious) epoch pre-killing. Means and standard errors for spectral variables for baseline and knock-down periods are listed in Table 5.7.

Table 5.7 EEG summary statistics (mean, SE and N) for spectral variables for each killing treatment at two periods pre-killing; baseline (awake/conscious) and knock-down anaesthetic (unconscious).

State/Period	Device	PTOT		F50		F95		N*
		Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	
Baseline	MCD	859.0	129.2	23.5	1.1	66.5	1.9	63
	MARM	616.1	84.4	24.6	1.5	74.8	1.2	48
	NMCD	653.8	48.3	21.0	1.1	70.8	1.3	66
After ‘knock-down’	MCD	3733.0	620.8	7.2	0.7	25.4	1.6	21
	MARM	4611.0	1218.0	7.0	0.3	24.6	3.2	6
	NMCD	3660.0	1211.0	6.7	0.4	22.7	1.9	22

* *Number of epochs varies as not every measure was available for every bird; baseline measures are based on three epochs per bird.*

Summary statistics were calculated for the first time to F50 < 12.7 Hz (maximum of unconsciousness range) and F50 < 6.8 Hz (mean of unconsciousness range) and are listed in Table 5.8. Killing treatment had an effect on first time to F50 < 12.7 Hz (see Table 5.9 for GLMM modelling results). MCD was associated with significantly the shortest F50 < 12.7 Hz latency post-killing (2.6 ± 1.5 s), compared to NMCD (3.1 ± 1.6 s) and MARM (3.5 ± 2.6 s); however there was no significant difference between latencies for NMCD and MARM. When any of the devices performed optimally, this significantly reduced the time to F50 < 12.7 Hz (“device success” means: Yes = 1.5 ± 0.4 s; and No = 5.6 ± 1.7 s). Bird type also had an effect, with layer type birds (hens and pullets) exhibiting longer F50 < 12.7 Hz timings than slaughter-age broilers (means: 5.3 ± 1.6 s; 2.4 ± 0.7 s, respectively). The three interactions of killing treatment with device success, bird type or bird age were all significant (Figure 5.4).

Killing treatment also had an effect on the first time to F50 < 6.8 Hz (see Table 5.9), with the MARM showing significantly longer time to F50 < 6.8 Hz compared to NMCD and MCD, which were not significantly different from one another. As with F50 < 12.7 Hz, latencies to F50 < 6.8 Hz were significantly shorter when the device application was optimal (Device success means: Yes = 2.3 ± 1.4 s; and No = 4.3 ± 2.2 s). Bird type also had a significant effect with shorter latencies for slaughter-age broilers (mean 4.7 ± 2.1 s) compared to layer type birds (hens and pullets) (mean 5.7 ± 1.5s). The remaining fixed effects (e.g. bird weight, bird age, and interactions) were not significant.

Table 5.8 EEG summary statistics (mean, standard error (SE), minimum (Min), maximum (Max) and number of birds (N)) for time to unconsciousness thresholds (F50 < 12 Hz and F50 < 6.8 Hz); first time to isoelectric; and last time not isoelectric for all successful kills in implanted birds for each killing treatment

	Killing treatment	Time post-kill (s) Mean	SE	Min	Max	N[$]
First time to F50 < 12.7 Hz	MCD	2.6	1.5	1	32	17
	MARM	3.5	2.6	1	20	13
	NMCD	3.1	1.6	1	11	16
First time to F50 < 6.8 Hz	MCD	3.2	0.3	1	32	19
	MARM	3.5	0.3	1	40	13
	NMCD	3.1	0.3	1	16	15
First time to isoelectric*	MCD	41.8	6.3	11	80	12
	MARM	72.0	16.1	20	170	9
	NMCD	46.3	6.0	8	85	14
Last time not isoelectric*	MCD	39.2	5.2	2	65	13
	MARM	43.9	8.1	4	95	15
	NMCD	21.5	5.2	10	46	10

** Threshold (PTOT < 170 mV and F50 > 17 Hz) automatically calculated.*
[$] Number of epochs varies as not every measure was available for every bird.

Table 5.9 GLMM analyses output for modelling latencies to unconsciousness and isoelectric EEG through calculated spectral variable thresholds. Significant *P* values (*P* < 0.05) are underlined.

Fixed Effects	df	First time to F50 < 12.7 Hz *F*	*P*	First time to F50 < 6.8 Hz *F*	*P*	First time to isoelectric *F*	*P*	Last time NOT isoelectric *F*	*P*
Killing treatment	2	3.83	<u>0.022</u>	4.24	<u>0.022</u>	23.64	<u><0.001</u>	6.20	<u>0.002</u>
Device success	1	8.66	<u>0.003</u>	8.75	<u>0.005</u>	17.12	<u><0.001</u>	0.52	0.470
Bird type	1	3.88	<u>0.049</u>	7.17	<u>0.011</u>	1.29	0.273	0.28	0.595
Bird age	1	0.47	0.495	1.01	0.322	4.23	0.053	0.35	0.555
Bird weight	1	0.02	0.883	0.89	0.350	0.10	0.881	0.75	0.388
Killing treatment. device success	2	9.73	<u><0.001</u>	2.68	0.081	0.12	0.883	1.42	0.242
Killing treatment . bird type	2	3.61	<u>0.027</u>	2.80	0.073	4.23	<u>0.031</u>	2.55	0.078
Killing treatment .bird age	2	7.39	<u><0.001</u>	2.68	0.081	0.01	0.952	1.85	0.157
Killing treatment .bird weight	2	0.28	0.759	0.89	0.417	0.00	1.000	1.90	0.149

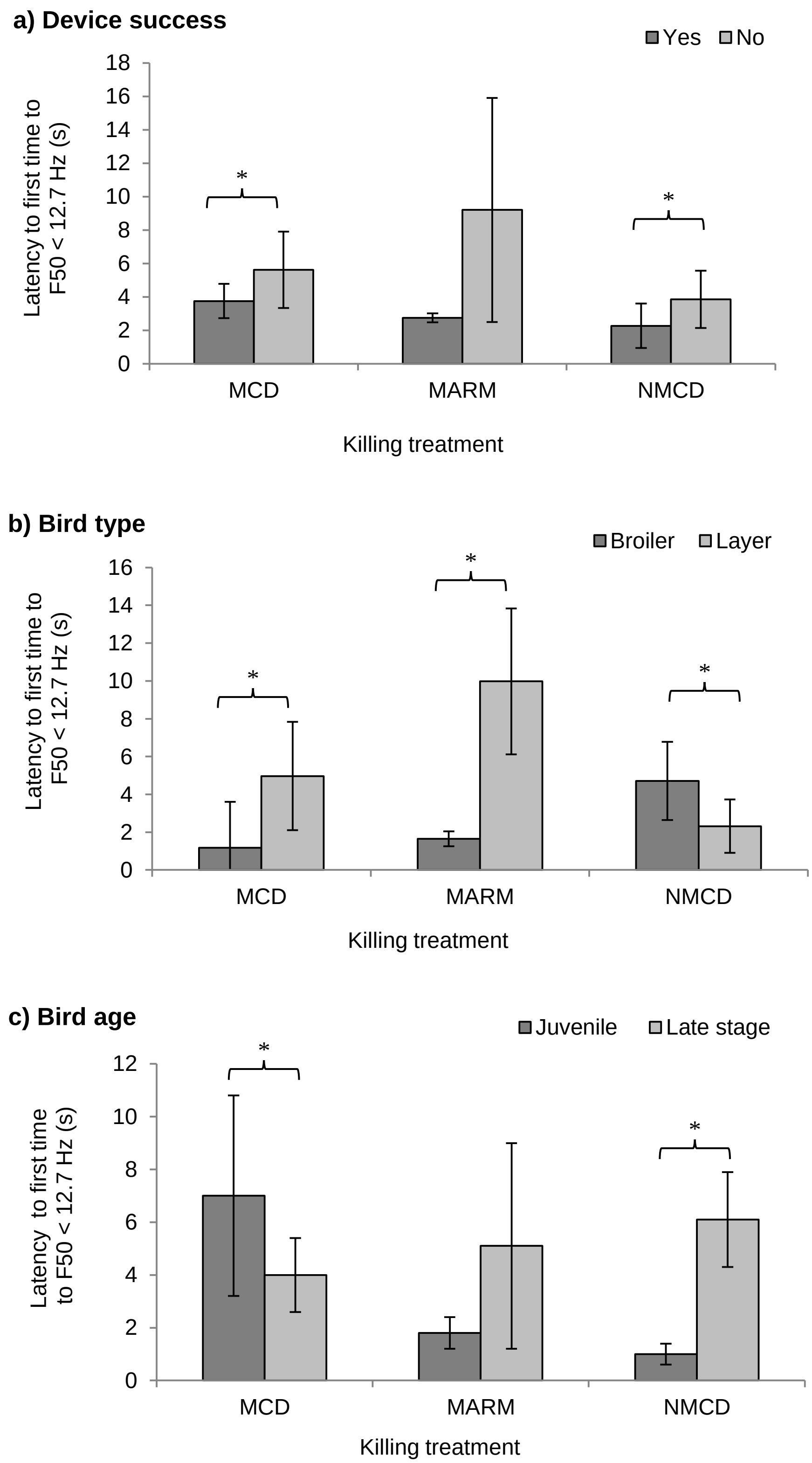


Figure 5.4 Effects on mean latencies to F50 < 12.7 Hz in the EEG post-killing for significant interactions between killing treatment and (a) device success; (b) bird type; and (c) bird age (juvenile = layer pullet; late stage = slaughter age broiler and laying hen). * indicates a significant difference between groups.

The first time to isoelectric and last time not isoelectric were calculated (Table 5.8) in order to provide an estimates of when brain death occurred and reduce the risk of missing data elongating the calculated summary statistic durations, as more 2 s epochs become unusable as the biological contribution to the EEG trace diminishes (during the transition to isoelectric signal). Killing treatment had an effect on both measures, with the MARM having the longest latencies for both (first time = 72.0 ± 16.1 s; last time = 43.9 ± 8.1 s) compared to the MCD (first time = 41.8 ± 6.3 s; last time = 39.2 ± 5.2 s) and the NMCD (first time = 46.3 ± 6.0 s; last time = 21.5 ± 5.2 s). For first time to isoelectric there was no significant difference between MCD and NMCD however for last time not isoelectric NMCD had significantly the shortest timing compared to both MCD and MARM. No other factors had an effect on last time not isoelectric. Device success had an effect on first time to isoelectric with ’device success = yes’ resulting in shorter durations (mean = 2.3 ± 1.4 s), compared to ’device success = no’ (mean = 4.3 ± 2.2 s). The only other factor which had a significant effect was an interaction between killing treatment and bird type, where there was no difference between bird types for MCD, however for the MARM, layers had significantly longer latencies compared to broilers, but for NMCD broilers had longer latencies then layers (Figure 5.5).

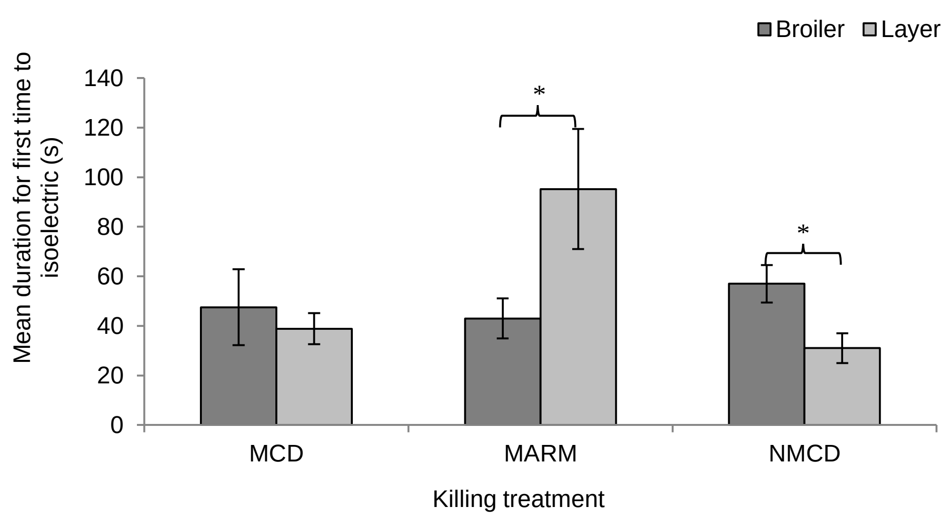


Figure 5.5 Effect of the interaction between killing treatment and bird type on the first time to isoelectric EEG (s). * indicates a significant difference between groups.

Assessment of continuous consciousness states indicate whether the killing method caused unconsciousness in the birds and maintained it until brain death. Figures 5.6 – 5.8 demonstrate the time series of mean PTOT and F50 for all killing treatments which had EEG measurements taken. All three killing treatments caused a sharp increase in PTOT after application, although the timing of the peak and magnitude was killing method specific. For MCD the peak occurred within the 1-3 s interval, with a mean peak of 41,926.0 ± 41,281.0 mV. For NMCD, the peak was delayed and longer lasting, occurring across the 7 - 9 s and 9 - 11 s intervals, with a mean of 47,732.0 ± 17,632.0 mV. However, for the MARM, two PTOT peaks occurred post method application; the first occurring between 4-5 s interval (mean = 14,111.0 ± 12,329.0 mV) and the second 9-11 s interval (mean = 13,135.0 ± 8340.9 mV), both were considerably lower in power compared to the peaks of MCD and NMCD.

Figure 5.6 demonstrates that the majority birds (N = 19) which had MCD applied to them appeared to remain unconsciousness (means and SE below unconsciousness threshold (F50 ≤ 12.7 Hz)) from the point of application for 65.6% of time intervals (21/32 intervals), and that 63.2% of birds were ECI within 1 minute post method application (12/19 birds). Only 31.3% of time intervals were below the mean unconscious frequency threshold (F50 ≤ 6.8 Hz). The mean spectral variables post method application were PTOT = 1722.9 ± 304.6 mV and F50 = 9.1 ± 1.6 Hz. Figure 5.7 shows the time series for spectral variables for birds that underwent the NMCD treatment (N = 17) and demonstrates that the majority of birds remained in the unconsciousness threshold (F50 ≤ 12.7 Hz) until brain death (95.7% of time intervals), which all birds reached by the 42 s interval. Only 34.8% of time intervals were below the mean unconscious threshold (F50 ≤ 6.8 Hz).The mean spectral variables post method application were PTOT = 6296.4 ± 1113.1 mV and F50 = 7.8 ± 1.4 Hz.

The time series for spectral variables for the MARM method are shown in Figure 5.8 (N = 11) and shows most birds remained below the unconsciousness threshold (F50 ≤ 12.7 Hz) from the point of application for 81.3% of time intervals (26/32 intervals). However, only 45.5% of birds reached ECI within 1 minute post method application (5/11 birds). The mean spectral variables post method application were PTOT = 2544.9 ± 449.9 mV and F50 = 8.5 ± 1.5 Hz.

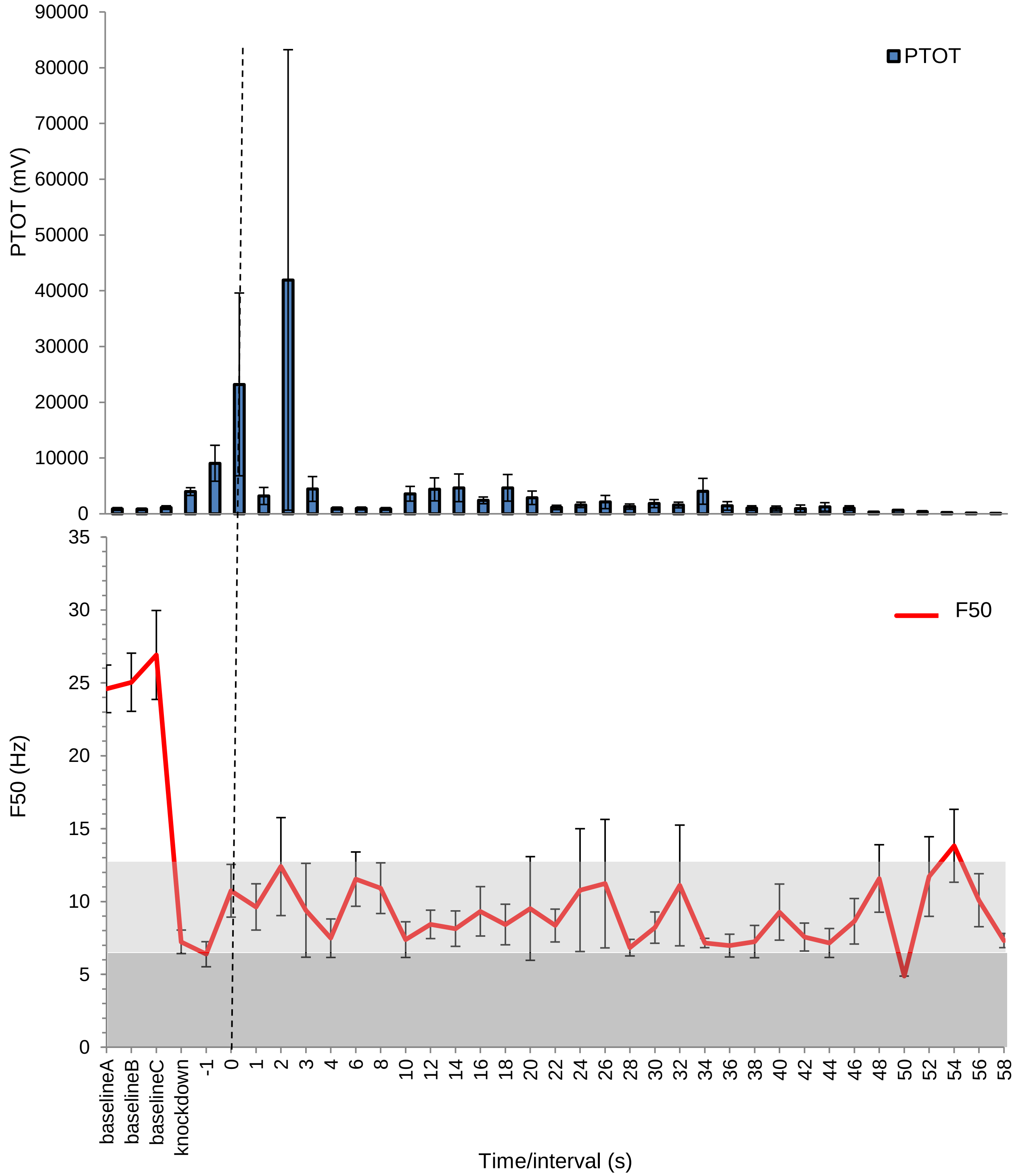


Figure 5.6 Time series for MCD of mean (±SE) PTOT and F50 spectral variables from baseline, to knock-down (anaesthetised), "kill" (application of killing method at 0 s), and every 2 s post-application for 1 minute. Number of epochs per time interval varies from 7 – 17 epochs (total N for MCD = 19). The F50 unconsciousness thresholds are indicated on the graph: F50 of 6.8 to < 12.7 Hz = light grey shading; F50 of 0.0 to < 6.8 Hz = dark grey shading. The dotted black line indicates where the killing method application occurred. Once birds were identified as brain dead they were removed from the graph time series in order to prevent data skewing as the biological relevance of the EEG trace reduced and the "mains hum" dominated the signal (Lowe et al., 2007; McKeegan et al., 2013b).

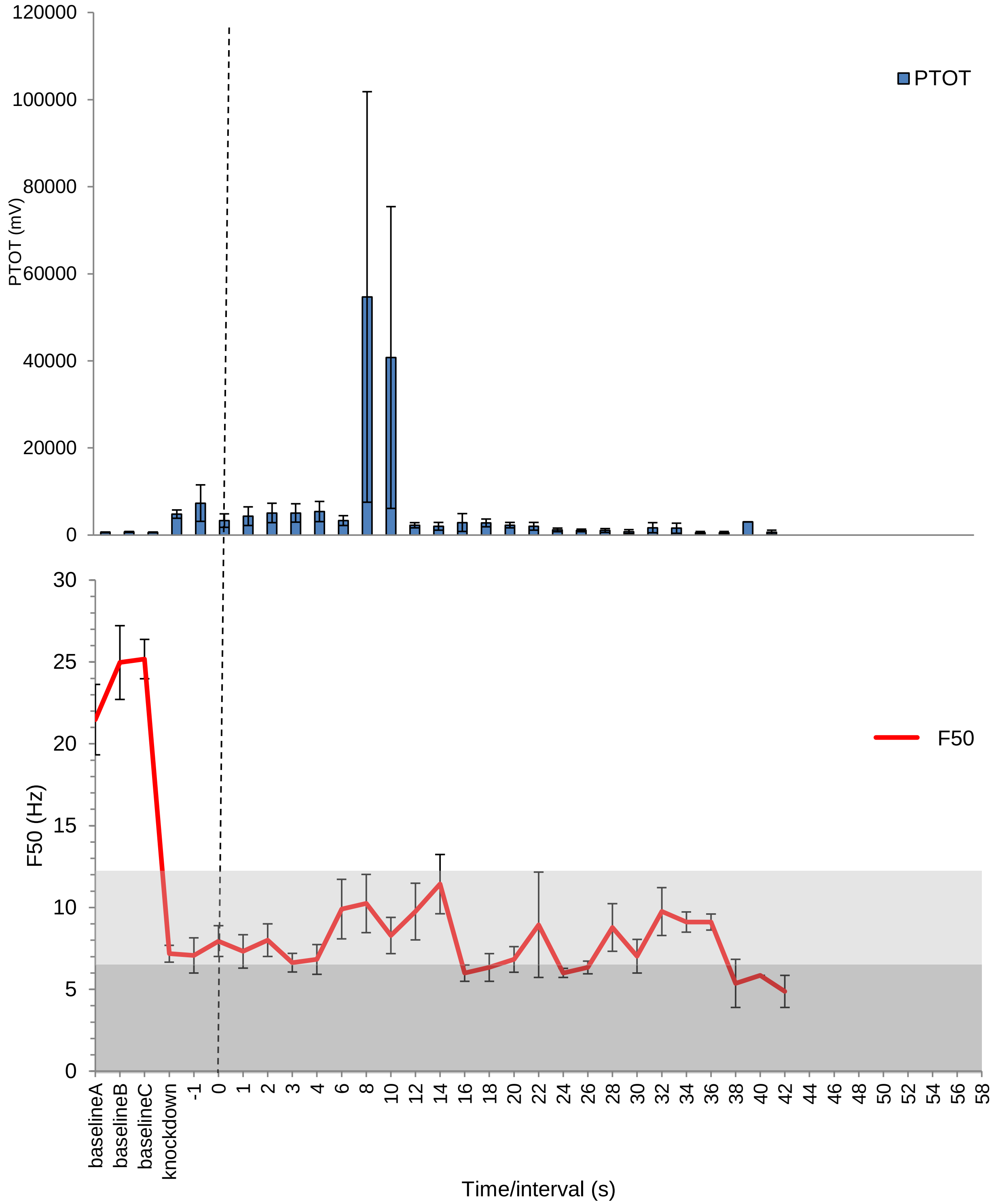


Figure 5.7 Time series for NMCD of mean (±SE) PTOT and F50 spectral variables from baseline, to knock-down (anaesthetised), "kill" (application of killing method at 0 s), and every 2 s post-application for 1 minute. Number of epochs per time interval varies from 4 – 16 epochs (total N for NMCD = 17). The F50 unconsciousness thresholds are indicated on the graph: F50 of 6.8 to < 12.7 Hz = light grey shading; F50 of 0.0 to < 6.8 Hz = dark grey shading. The dotted black line indicates where the killing method application occurred. Once birds were identified as brain dead they were removed from the graph time series in order to prevent data skewing as the biological relevance of the EEG trace reduced and the "mains hum" dominated the signal (Lowe et al., 2007; McKeegan et al., 2013b).

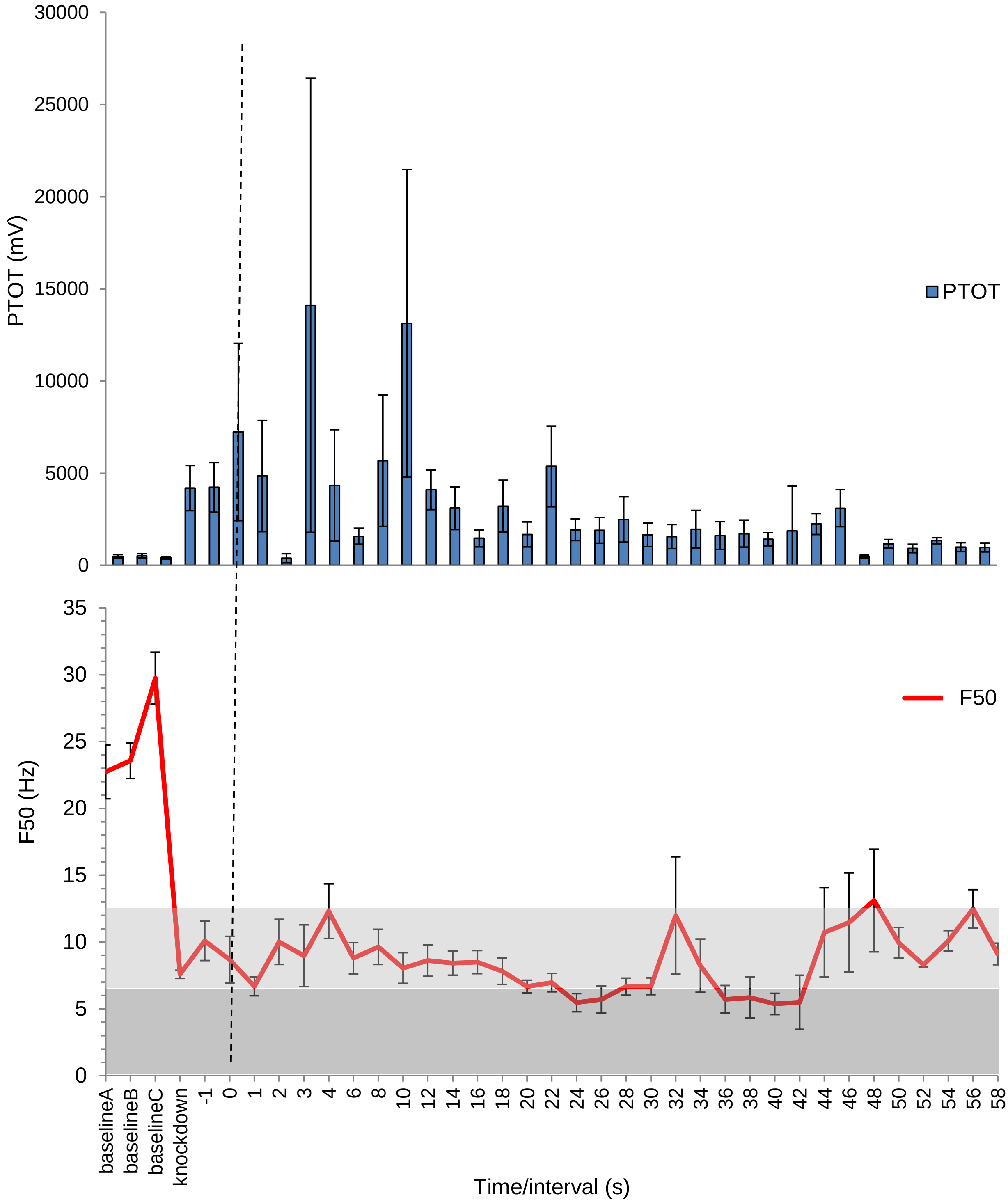
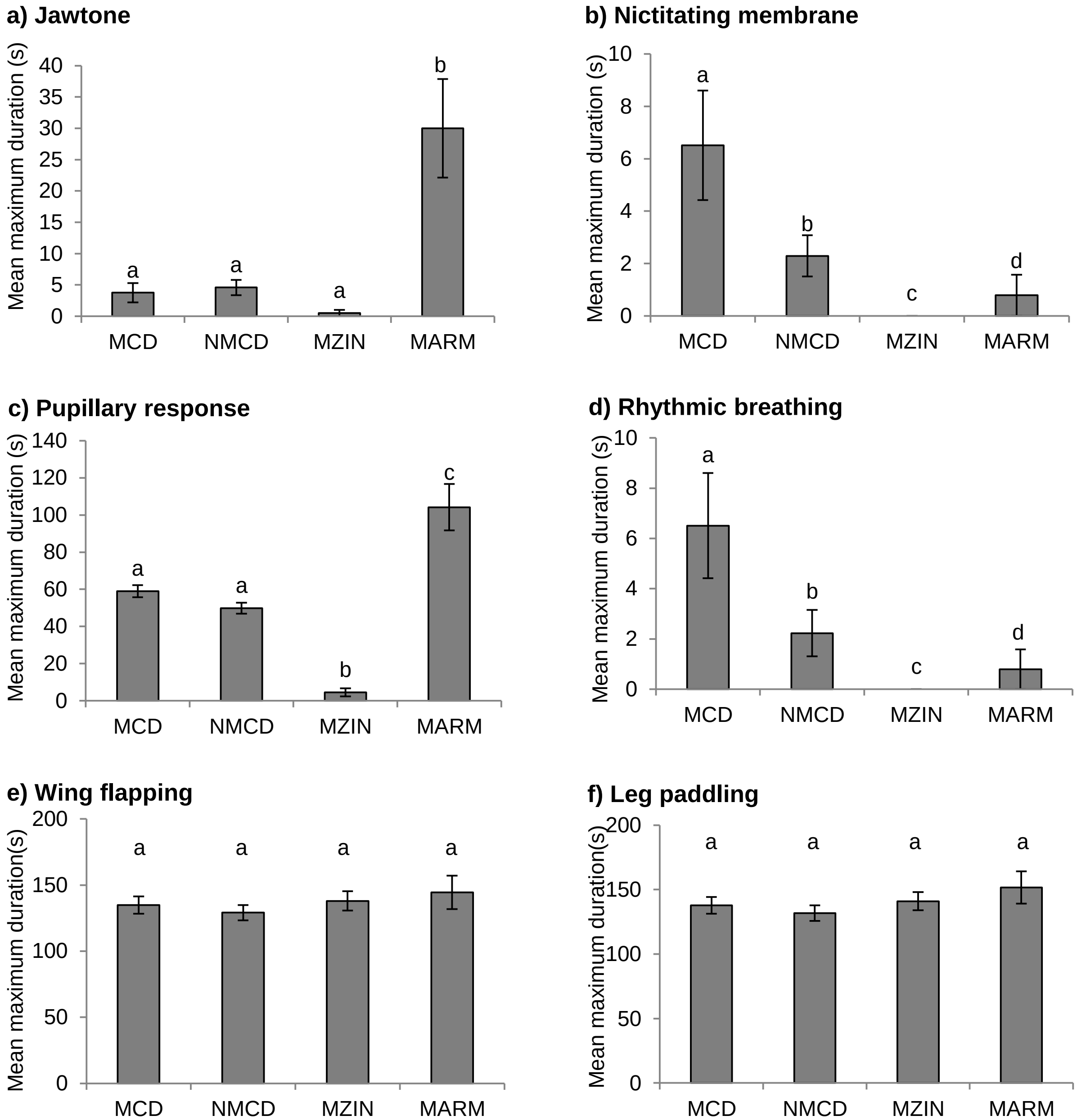


Figure 5.8 Time series for MARM of mean (±SE) PTOT and F50 spectral variables from baseline, to knock-down (anaesthetised), "kill" (application of killing method at 0 s), and every 2 s post-application for 1 minute. Number of epochs per time interval varies from 4 – 9 epochs (total N for MARM = 11). The F50 unconsciousness thresholds are indicated on the graph: F50 of 6.8 to < 12.7 Hz = light grey shading; F50 of 0.0 to < 6.8 Hz = dark grey shading. The dotted black line indicates where the killing method application occurred. Once birds were identified as brain dead they were removed from the graph time series in order to prevent data skewing as the biological relevance of the EEG trace reduced and the "mains hum" dominated the signal (Lowe et al., 2007; McKeegan et al., 2013b).

5.3.4 Behavioural Observations

Comparisons of mean maximum durations for all reflexes and behaviours across killing treatments are shown in Figure 5.9 and results of the GLMM analyses are shown in Table 5.10. Killing treatment had a significant effect on jaw tone; nictitating membrane; pupillary and rhythmic breathing; but not on cloacal movement.

Despite the significant overall effect of killing treatment on durations of jaw tone, the only significant differences were between the MARM and the other treatments, and not between the MZIN, MCD and NMCD. Across all birds, 77.6% never showed jaw tone following application of the killing treatments; however of the birds that did, the descriptive statistics for jaw tone duration observed were: MARM (N = 16) mean = 33.8 ± 8.4 s, min = 15.0 s, max = 150.0 s; MCD (N = 12) mean = 23.8 ± 7.5 s, min = 15.0 s, max = 105.0 s; NMCD (N = 15) mean = 22.0 ± 2.9 s, min = 15.0 s, max = 45.0 s; and MZIN (N = 1) mean = 15.0 ± 0.0 s, min = 15.0 s, max = 15.0 s. There was an interaction between killing treatment and bird age (Figure 5.10), with no differences related to age for MCD, NMCD and the MZIN, however in the MARM treatment, younger birds showed significantly greater durations of jaw tone compared to older (i.e. late production stage) birds. Device success, bird type, bird age, bird weight, and all other interactions were not significant.



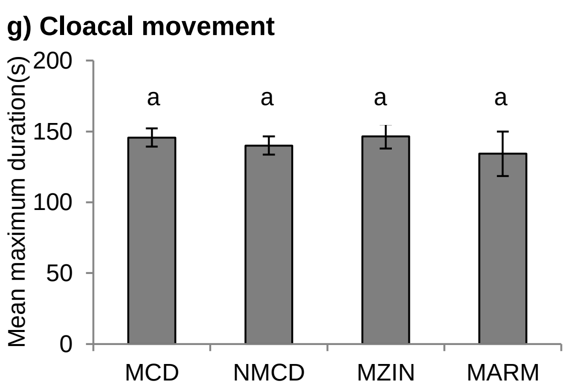


Figure 5.9 Graphical summaries of the mean maximum durations (s) across the three kill treatments for the cranial reflexes and death related behaviours: a) jaw tone; b) nictitating membrane; c) pupillary response; d) rhythmic breathing; e) wing flapping; f) leg paddling; and g) cloacal movement. Please note that the y axes scales vary. No common superscript indicates that there is a significant difference between the groups.

Table 5.10 GLMM analysis output of the minimum models for maximum reflex and behaviour durations in response to killing treatment (N = 196).

Factor	df	Jaw tone		Nictitating membrane		Pupillary		Rhythmic breathing		Wing flapping		Leg paddling		Vent movement	
		F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Killing treatment	3	21.11	<0.001	2.91	0.036	59.5	<0.001	2.91	0.036	0.63	0.595	0.94	0.424	0.37	0.778
Bird type	1	0.43	0.512	2.77	0.099	14.08	<0.001	2.75	0.099	39.55	<0.001	37.47	<0.001	32.13	<0.001
Bird age	1	0.62	0.431	0.42	0.518	0.09	0.767	0.42	0.518	0.56	0.454	2.01	0.158	0.15	0.695
Bird weight	1	0.62	0.433	4.98	0.027	0.57	0.453	4.98	0.027	2.26	0.134	0.08	0.781	9.94	0.002
Killing treatment .bird type	3	3.61	0.086	1.7	0.999	2.83	0.039	1.6	0.166	0.71	0.546	1.07	362	1.41	0.241
Killing treatment .bird age	3	5.76	0.012	0.27	0.517	3.67	0.013	0.27	0.849	0.88	0.454	0.91	0.438	1.47	0.224
Killing treatment .bird weight	3	1.1	0.349	0.95	0.420	6.98	<0.001	0.95	0.42	0.37	0.771	1.11	0.345	1.4	0.245

Note: Significant P values are underlined.

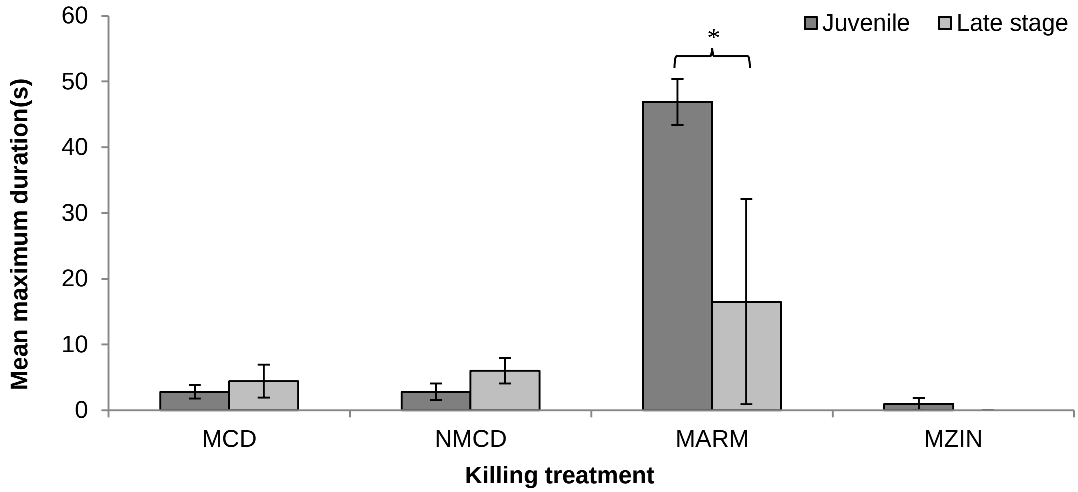


Figure 5.10 Interaction between grouped bird ages and killing treatment for the mean maximum duration (s) of jaw tone post method application. Juvenile = broiler chicks and layer pullets; and late stage = broilers (slaughter age) and laying hens. * indicates a significant difference between groups.

For nictitating membrane reflex, apart from killing treatment, only bird weight had an effect, with all other factors and interactions having no effect. Bird weights and nictitating membrane durations were positively correlated (r = 0.201, P = 0.005), with durations being longer for heavier birds compared to lighter ones.

Bird type had an effect on the pupillary reflex duration, with broilers exhibiting significantly shorter durations compared to layers (means: broilers = 42.0 ± 3.7 s; layers = 59.2 ± 3.8 s). There was an interaction between bird type and killing treatment with broilers having lower pupillary durations compared to layers for MCD, NMCD and MARM (Figure 5.11). However, MZIN broilers had significantly longer durations compared to layers (6.2 ± 0.0 s and 2.3 ± 0.0 respectively). In NMCD, MCD and MZIN treatments, the interaction between killing treatment and bird age was not significant (Figure 5.12), but for the MARM the pupillary reflex durations were significantly longer in juvenile birds compared to older birds. The interaction effect between killing treatment and bird weight showed there was no effect for MCD, MZIN and NMCD, however, for MARM heavier birds had significantly shorter pupillary reflex durations (mean = 62.1 ± 12.0 s) compared to lighter birds (mean = 120.0 ± 30.0 s).

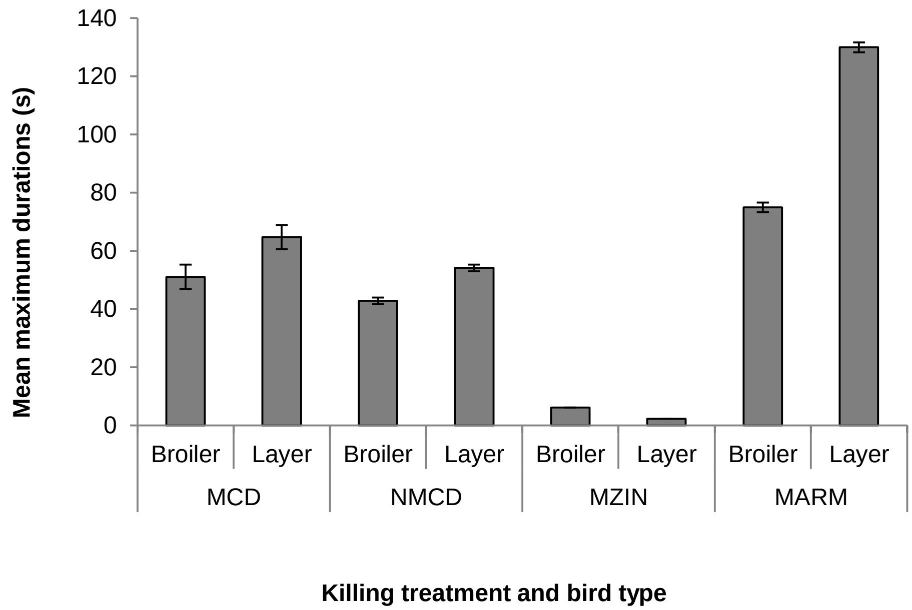


Figure 5.11 Interaction effects between killing treatment and bird type on durations for the pupillary reflex. Only data from successful kills are shown.

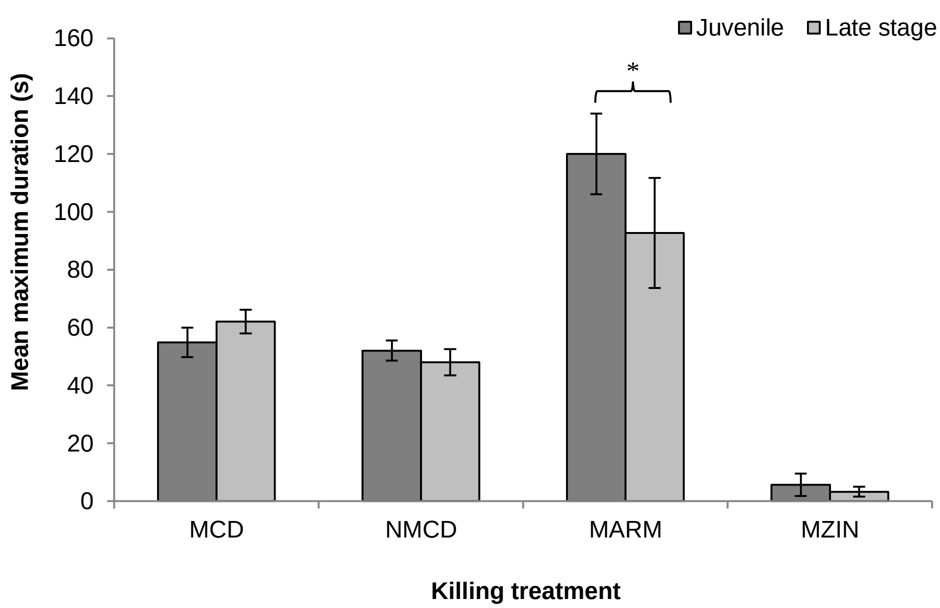


Figure 5.12 Interaction effects between killing treatment and grouped bird age (i.e. juvenile / late stage production) on durations for the pupillary reflex. Only data from successful kills are shown. * indicates a significant difference between groups.

Apart from the killing treatment, only bird weight had an effect on rhythmic breathing, with all other factors and interactions having no effect. Bird weights and rhythmic breathing durations were positively correlated (r = 0.201, P = 0.006), with breathing lasting longer in heavier birds (mean = 7.2 ± 2.6 s) compared to lighter birds (mean = 0.9 ± 0.9 s).

The durations of wing flapping and leg paddling post killing-treatment application were affected only by bird type, no other factors, including killing treatment, or interactions had a significant effect. In both cases broilers exhibited significantly shorter durations compared to layers (Figure 5.13).

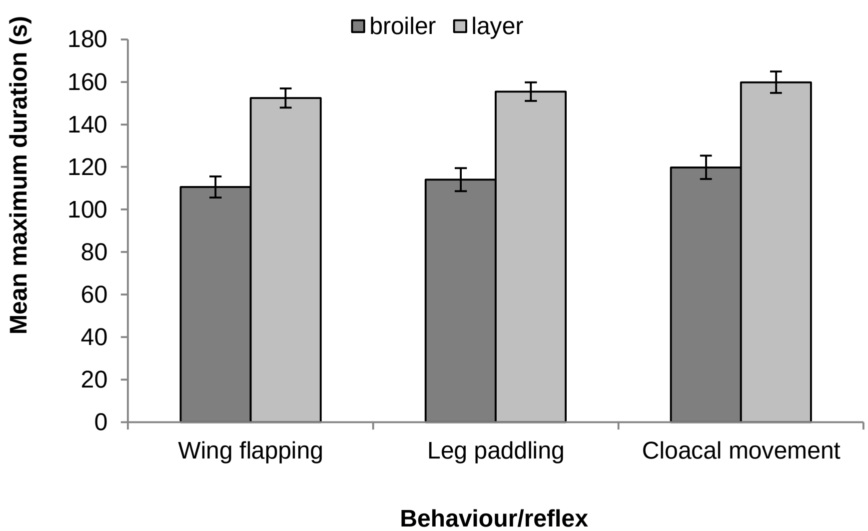


Figure 5.13 Comparison of wing flapping, leg paddling, and cloacal movement durations across all killing treatments by bird type (broiler / layer).

The duration of cloacal movement was affected by bird type (Figure 5.13) and bird weight, with broilers having shorter durations compared to layers. Cloacal movement duration and bird weight were negatively correlated (r = - 0.180, P = 0.012), with heavier birds exhibiting shorter durations (mean = 125.0 ± 6.9 s) for cloacal movement compared to lighter birds (mean = 153.3 ± 6.7 s).

5.3.5 Correlation analysis between EEG and reflex and behaviour data

The were no significant correlations between reflex durations post-application (rhythmic breathing, pupillary, nictitating membrane, jaw tone and the duration for all behaviours) and the calculated first time to the two unconsciousness thresholds, first time to isoelectric and last time not isoelectric (Table 5.11).

Table 5.11 Correlation matrix between maximum reflex and behaviour durations post killing and the calculated first time to the two unconsciousness thresholds, first time to isoelectric and last time not isoelectric.

Consciousness state / EEG thresholds		Maximum reflex/behaviour duration				
		Rhythmic breathing	**Pupillary**	**Nictitating membrane**	**Jaw tone**	**All reflexes and behaviours**
First time to F50 < 6.8 Hz	*r*	-0.249	0.038	-0.249	-0.153	-0.030
	P	0.219	0.853	0.219	0.454	0.881
First time to F50 < 12.7 Hz	*r*	-0.284	0.104	-0.284	-0.048	-0.105
	P	0.158	0.610	0.158	0.812	0.608
First time to isoelectric	*r*	-0.062	-0.065	-0.062	-0.083	-0.333
	P	0.760	0.749	0.760	0.686	0.095
Last time not isoelectric	*r*	-0.002	-0.048	-0.001	-0.133	-0.331
	P	0.993	0.815	0.993	0.516	0.098

5.3.6 Post-mortem evaluations

5.3.6.1 Cervical dislocation methods: MCD and NMCD

For successfully-killed birds (MCD = 76/76 birds; NMCD = 72/75 birds), post-mortem results showed that all birds (100%) had their necks fully dislocated and their spinal cord severed, with no intra-vertebrae damage, irrespective of cervical dislocation method. There was no difference in the location of the dislocation point between the two methods ($F_{1,152}$ = 0.05, P = 0.816), with the majority of birds receiving a C0-C1 dislocation (Figure 5.14). MCD resulted in the lowest dislocation level recorded (C4-C5), which occurred in two birds, a layer hen (1.5 kg) and a slaughter age broiler (3.2 kg). Dislocation point means were calculated by converting vertebral levels to a numerical category (e.g. C0-C1 = 1; C1-C2 = 2; C2-C3 = 3; C3-C4 = 4; and C4-C5 = 5). Age at killing ($F_{1,152}$ = 10.18, P = 0.002) and neck gap ($F_{1,152}$ = 11.61, P < 0.001) had a significant effect on dislocation point, with older birds (mean = 1.51 ± 0.09) being more likely to have a lower dislocation compared to younger birds (mean = 1.05 ± 0.03). Larger neck gap sizes (between two dislocated vertebrae) were observed in higher dislocation levels compared to lower dislocation levels (Figure 5.15). Bird type, bird weight and all interactions did not have significant effects.

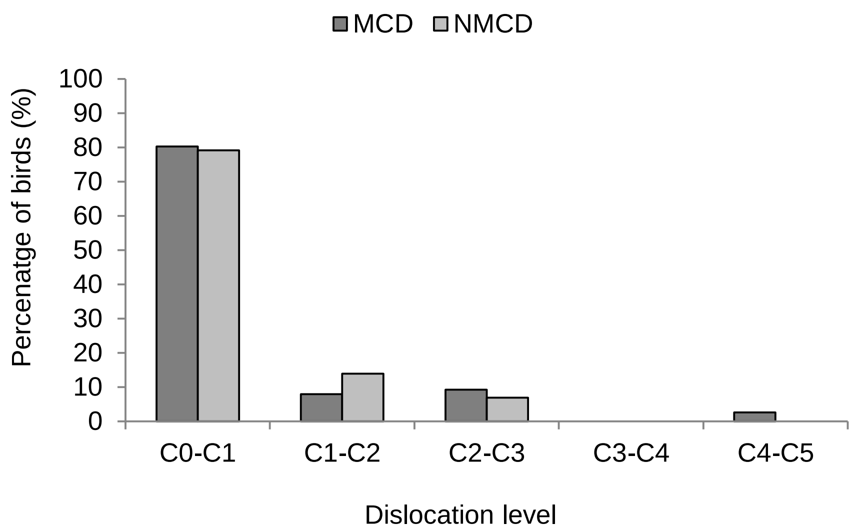


Figure 5.14 The distribution of birds across the range of dislocation levels produced by MCD and NMCD.

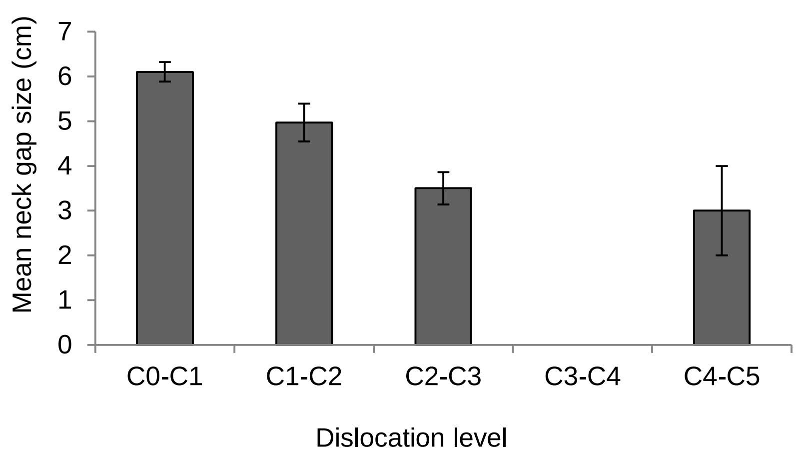


Figure 5.15 Mean (±SE) neck gap sizes across the different dislocation levels.

The size of gap between the dislocated vertebrae was significantly affected by killing treatment ($F_{1,152}$ = 5.59, P = 0.022), with NMCD (mean = 6.6 ± 0.3 cm) more likely to result in a larger neck gap size compared to MCD (mean = 5.3 ± 0.3 cm). Bird type ($F_{1,152}$ = 8.92, P = 0.004) and bird age ($F_{1,152}$ = 13.92, P < 0.001) also had an effect with layers and younger birds having larger neck gap sizes compared to broilers and adults (bird type: layers = 5.9 ± 0.3 cm; broiler = 5.5 ± 0.3 cm; bird age: adult = 5.4 ± 0.3 cm; juvenile = 6.1 ± 0.2 cm). All other fixed effects and interactions did not have an effect on neck gap size.

As a result of the method application the skin was broken in 23.6% of birds undergoing NMCD and 13.2% of birds for MCD, although this difference was not significant ($F_{1,152}$ = 2.55, P = 0.112). Bird type, age, weight and their interactions were not significant in relation to whether the skin was broken or not. In both methods, the majority of birds sustained a subcutaneous hematoma (MCD = 100.0%; NMCD = 98.6%) and severe muscle damage and tearing to the muscle in the neck (MCD = 100.0%; NMCD = 98.6%) Most birds also had one or both carotid arteries severed (MCD = 72.4%; NMCD = 87.5% - Figure 5.16), although NMCD was significantly more likely to sever one or both arteries than MCD ($F_{1,152}$ = 11.05, P < 0.001). Bird weight ($F_{1,152}$ = 18.25, P < 0.001) had an effect on the number of carotid arteries severed (Figure 5.17), with both carotid arteries more likely to be severed in lighter birds. Larger neck gaps sizes were more likely to result in one or both carotid arteries being severed ($F_{1,152}$ = 32.19, P < 0.001) (Figure 5.18). Bird type, bird age, EEG implant and all interactions did not have an effect on damage to the carotids.

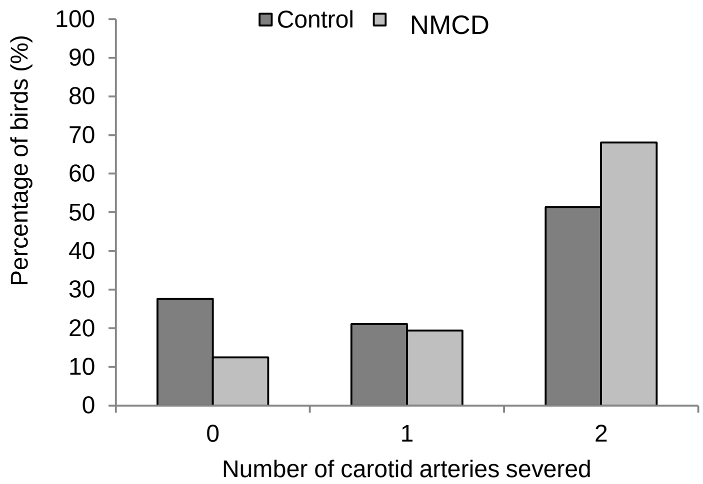


Figure 5.16 Percentage of birds killed by MCD and NMCD, which resulted in 0, 1 or 2 carotid arteries being severed.

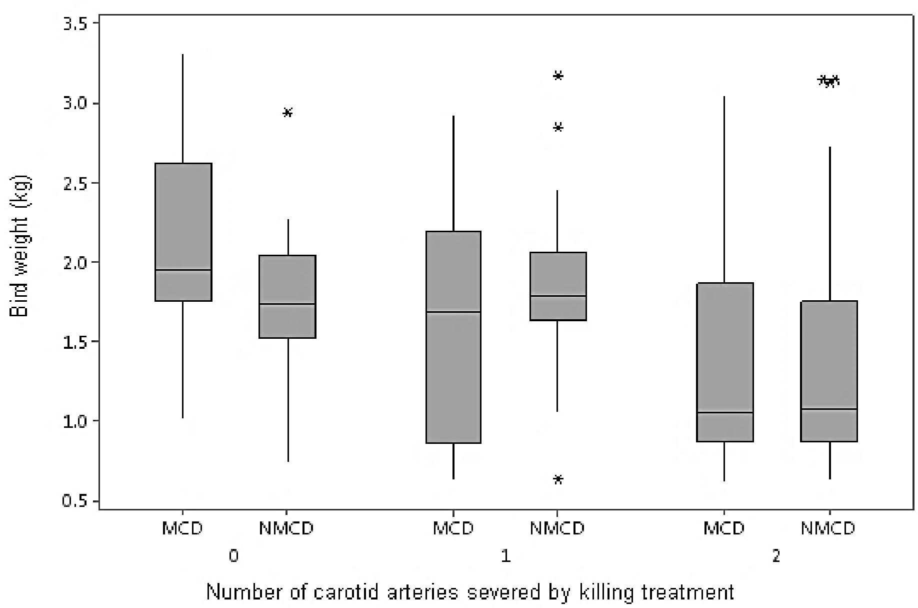


Figure 5.17 The effect of bird weight (kg) on the number of carotid arteries severed as a result of the application of MCD and NMCD killing treatments.

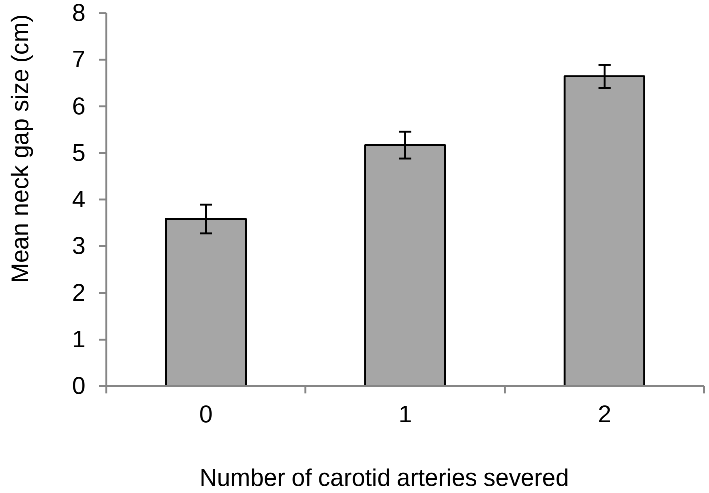


Figure 5.18 Mean neck gap sizes recorded in relation to the number of carotid arteries severed for NMCD and MCD successfully killed birds.

5.3.6.2 Brain trauma methods: MARM and MZIN

Kill success had a significant effect on a number of post-mortem measures for both brain-trauma killing treatments, both of which were less successful than dislocation methods (successful kills: MARM = 19/39 birds; MZIN = 30/40 birds) (Table 5.12). The majority of measures were more likely to occur at all when the kill was successful for either treatment. There were a few exceptions, for example, for the MARM, both left and right forebrain damage occurred more in unsuccessfully killed birds and for the MZIN more birds suffered subcutaneous hematomas when un-successfully killed. Following successful kills, all birds exhibited skin breaks, external bleeding and damage to the skull, irrespective of killing treatment.

Table 5.12 Percentage of birds for which the post-mortem measure was present, related to killing treatment (MZIN and MARM) and whether the kill was successful or not. Degrees of freedom = 1 for all measures. Significant *P* values (*P* < 0.05) are underlined.

Post mortem measure	**Percentage of birds observed**				***P value***
	MZIN		**MARM**		
	Kill Success 'Y'	**Kill Success 'N'**	**Kill Success 'Y'**	**Kill Success 'N'**	
Skin broken	100.0	90.0	100.0	95.0	0.088
External bleeding	100.0	80.0	100.0	90.0	0.062
Subcutaneous hematoma	90.0	100.0	89.5	85.0	0.074
Skull damage	100.0	80.0	100.0	85.0	<u>0.044</u>
Brain cavity hematoma	100.0	60.0	94.7	50.0	<u>0.032</u>
Left forebrain damage	83.3	10.0	0.0	5.0	0.073
Right forebrain damage	83.3	0.0	0.0	10.0	<u>0.016</u>
Cerebellum damage	86.7	10.0	63.2	20.0	<u><0.001</u>
Midbrain damage	96.7	10.0	10.5	5.0	<u>0.018</u>
Brain stem damage	3.5	0.0	84.2	0.0	<u><0.001</u>

The location of the skull damage and/or penetration was affected by kill success ($F_{1,78}$ = 5.66, P = 0.016) and killing treatment ($F_{1,78}$ = 7.10, P < 0.001). For successfully killed birds, the range of skull areas damaged was lower in both devices compared to unsuccessfully killed birds (Table 5.13).

Table 5.13 Distribution of skull damage and penetration sites for birds across the two killing treatments and related to whether the kills were successful or not.

Skull penetration and/or damage location*	**Percentage of birds observed in (%)**			
	MARM		**MZIN**	
	Kill Success 'Y'	**Kill Success 'N'**	**Kill Success 'Y'**	**Kill Success 'N'**
LF (left front)	0.0	0.0	0.0	0.0
CF (central front)	0.0	5.0	0.0	0.0
RF (right front)	0.0	5.0	0.0	0.0
LM (left mid)	0.0	5.0	0.0	10.0
CM (central mid)	0.0	0.0	33.3	0.0
RM (right mid)	0.0	0.0	10.0	10.0
LB (left back)	0.0	30.0	0.0	10.0
CB (central back)	100.0	20.0	50.0	40.0
RB (right back)	0.0	20.0	0.0	10.0
X (no skull damage)	0.0	15.0	0.0	20.0

** Refer to Figure 5.2 for descriptions of skull penetration/damage locations.*

In successfully killed birds, the majority of brain damage occurred in the cerebellum and brain stem after application of the MARM, while for the MZIN all brain regions except the brain stem were damaged (Table 5.12). Damage to all brain regions except for the cerebellum were significantly affected by the killing treatment (Table 5.14). Bird weight affected whether or not the right forebrain (N = 1.67 ± 0.17 kg; Y = 1.45 ± 0.12 kg) and brain stem (N = 1.45 ± 0.10 kg; Y = 1.77 ± 0.20 kg) were damaged. The location of skull damage and/or penetration had a significant effect on whether or not the cerebellum and midbrain were damaged, with the midbrain most likely to be damaged when the central mid and right mid areas were shot, while for the brain stem, the right back area was most likely to result in damage. Only four skull regions were damaged when the kills were successful, irrespective of killing treatment, focussing around the central and right sides of the skull and the mid and back regions (refer Figure 5.2). Damage to the central mid, right mid and right back regions of the skull always resulted in damage to the midbrain and brainstem. Bird type, bird age and all interactions did not have significant effects on damage to individual brain regions.

Table 5.14 Results of GLMM analysis of damage to each brain region (binomial Y/N data): left forebrain, right forebrain, cerebellum, midbrain and brain stem for successful kills only (N = 49).

Factors	df	Left forebrain damage		Right forebrain damage		Cerebellum damage		Midbrain damage		Brain stem damage	
		F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Killing treatment	1,48	28.80	<0.001	22.36	<0.001	0.83	0.737	21.48	<0.001	35.55	<0.001
Bird type	1,48	0.46	0.502	0.36	0.553	0.11	0.606	0.21	0.686	0.05	0.819
Bird age	1,48	0.04	0.852	0.02	0.898	0.27	0.720	0.27	0.770	0.08	0.781
Bird weight	1,48	2.89	0.093	5.50	0.022	0.13	0.606	0.19	0.606	4.44	0.039
skull penetration location	8,48	1.84	0.179	0.08	0.774	5.86	0.016	4.03	0.042	0.13	0.721
Killing treatment. Bird type	1,48	0.00	0.961	0.21	0.650	1.84	0.175	0.05	0.812	0.00	0.993
Killing treatment. Bird age	1,48	0.01	0.937	0.00	0.971	2.82	0.093	1.12	0.196	2.83	0.097
Killing treatment. Bird weight	1,48	0.03	0.867	0.05	0.825	0.46	0.496	0.89	0.502	0.32	0.575

5.4 Discussion

The term ‘death’ in livestock and other domestic animals is poorly defined and is limited to cessation of respiration and cardiac activity, with the America Veterinary Medical Association confirming death “by examining the animal for cessation of vital signs, and consideration given to animal species and methods of euthanasia when determining the criteria for confirming death” (AVMA, 2007). In this experiment the killing efficacy of three novel mechanical killing treatments (MARM, MZIN, and NMCD) was investigated, and compared with the traditional MCD method. Efficacy was assessed in multiple ways (EEG activity, presence/absence of reflexes and post-mortem analysis) in order to accurately determine the time to unconsciousness and death for each treatment and make inferences about their effectiveness and humaneness.

Of the four methods tested, the control MCD was the most reliable, based on its 100% kill success rate, however the NMCD was the most successful mechanical method, with a marginally lower kill success rate of 96%. The NMCD was shown to be easy to use and adaptable to different bird types and ages, although was limited by bird weight, as three birds that weighed greater than 3.3 kg were not killed on the first attempt, requiring a second immediate attempt. However, this limitation was likely due to the operator’s strength, rather than the device itself and the same limitation would probably have applied to the MCD treatment had any of the birds in this group exceeded 3 kg (which they did not). The reliability of NMCD (e.g. 96%) concurred with other literature which reported similar high kill success rates of other mechanical cervical dislocation devices (e.g. Burdizzo and killing cone, (Erasmus et al., 2010a; Gregory and Wotton, 1990)). The decision to make a second attempt with the NMCD was made as a result of the operator not perceiving the “give” when the dislocation occurred; therefore the second attempt was immediately applied. The first attempt was likely to have caused trauma to the neck tissues and spinal cord through stretching (Dumont et al., 2001a; Shi and Pryor, 2002; Shi and Whitebone, 2006; Weir et al., 2002), although full dislocation was not achieved, primarily due to the birds’ heavier weight (> 3 kg). However, the second attempt achieved the dislocation with a perceived reduced effort, suggesting that the neck tissue was weakened as a result of the first attempt.

The primary reason for the lower kill success observed when using MARM and MZIN devices was the difficultly in aiming the devices. The top heaviness of the MZIN as well as the small size of the bird's head in ratio to the bolt muzzle, made it difficult to aim and balance the device on the head prior to firing. As the device was originally designed to kill rabbits (Pizzurro, 2009b), the significant difference in size (and ratio of head size and the bolt muzzle) and shape of the skull of the target species meant that, despite the modifications made (see Chapter 3.2.1), the device was still not a reliable method for despatching poultry. Other captive bolt devices (e.g. pneumatically-operated nailer gun – Draper Air Tools, UK; and Zephyr - NS 100A ¼ inch, Narrow Crown Stapler, Porter Cable, Jackson, TN) have been shown to be more successful, with 100% kills in poultry (Erasmus et al., 2010a; Raj and O'Callaghan, 2001). With the MARM, the main issue was the inadaptability of the device to different sizes and types of birds, even though in the analysis the effect was not significant. If a bird was slightly larger or smaller than the average bird for which the three individual head insertion cups (see Chapter 3.2.2) had been designed, there was no freedom to adapt the application of the device, which often resulted in the spike penetrating tissue in the wrong location and either minor or no brain damage occurring. This resulted in a very low kill success rate (< 50%), making the MARM an unreliable killing method for poultry.

In this experiment, the birds were anaesthetised prior to killing, yet still the MARM and MZIN performed badly. Therefore there was concern that when birds are awake and conscious they would be more likely to struggle when restrained, affecting the ability to aim the device successfully and making the results even poorer. Both devices also required two operators for their application, with one person holding the bird's body while the other positioned and applied the device. This is not practical in an on-farm situation where time and staff efficiency is at a premium; therefore the ideal is a killing method which requires only one operator, which is fulfilled by the NMCD and MCD.

Frustratingly, meaningful ECG data was not available after treatment application in the majority of birds, therefore time to cessation of heart rate activity was not available as an indicator of death (AVMA, 2007). There were also some issues with the recording of EEG data, with 38.4% of epochs unusable for a number of reasons (e.g. movement artefact). Similar issues have been reported in other experiments in which attempts have been made to the record EEG during and post-killing (Delorme and Makeig, 2004; Gwin et al., 2010; Lowe et al., 2007; McKeegan et al., 2013b). However, available EEG data did provide an insight into the time to brain death (i.e. when the trace became isoelectric) for the MARM, NMCD and MCD. NMCD was shown to result in the shortest duration to first time to isoelectric and last time not isoelectric compared to the only other mechanical device (MARM) measured, and although slightly numerically shorter durations for first time to isoelectric for the MCD, the difference between NMCD and MCD were not significant. The shorter duration to brain death may not be beneficial to the bird in terms of humaneness (unlike time to unconsciousness), but it does provide benefits in terms of practicality on-farm. Under EU legislation (European Council, 2009) and also required by several non-EU guideline documents (AVMA, 2007; Canadian Council on Animal Care in science, 2010) operators must confirm the success of a kill immediately post-application and must not move on or kill another individual until the present one is confirmed dead (i.e. loss of pupillary and nictitating membrane reflex, cessation of rhythmic breathing), therefore the shorter the duration is to brain death (Erasmus et al., 2010a; Facco et al., 2002; Sandercock et al., 2014), the quicker these measures will cease and the operator can continue with their duties. This could have indirect benefits to bird welfare, as if operators are forced to wait for less time, they may be more likely to wait and confirm death, and if necessary re-attempt a kill if it was unsuccessful, reducing the possibility that a severely injured bird will be left unattended for a prolonged period of time.

The duration of reflexes which are considered to indicate death (e.g. nictitating membrane, pupillary and rhythmic breathing, (Blackmore and Delany, 1988; Croft, 1961; Erasmus et al., 2010c; Gregory, 1991) did not correlate with the derived duration to isoelectric signal from EEG data. Both nictitating membrane and rhythmic breathing durations were considerably shorter for all killing treatments (all means < 10 s) compared to the mean durations to isoelectric (all > 20 s). Therefore, there is a risk that purely relying on these reflexes as an indication of death will incorrectly declare birds dead before this is true, as some electrical brain activity may still be occurring (although not necessarily implying consciousness), which could therefore impinge on their welfare. The short durations of nictitating membrane persistence seen here do not agree with other research (Erasmus et al., 2010a), in which mean durations for cervical dislocation methods ranged from 43 – 106 s (Erasmus et al., 2010a), while for captive bolt methods all means were 0 s (Erasmus et al., 2010a). The shorter durations observed in this study are believed to be due to the physiological trauma being caused as a result of the killing methods, which damage the brain stem (cortical control of reflexes) and can damage and supress blood supply to the eye (Blackman et al., 1986; McLeod et al., 1964), therefore affecting its behaviour. The Sevoflurane anaesthetic does not appear to have affected the durations adversely, since previously this anaesthetic prolonged such reflexes when birds were deeply anaesthetised (Sandercock et al., 2014; Smith, 1993). The anaesthetic method used here was rapid and had a short recovery time (mean ≤ 4 s) once inhalation of the gas ceased. Previous research has shown the importance of anaesthetic depth and types of anaesthetic chemicals used on their effects on reflexes (e.g. durations and suppression) (Haberham et al., 2008; Haberham et al., 2000).

Birds showed convulsive behaviours (e.g. leg paddling and wing flapping) post treatment application, and these behaviours continued for a prolonged period and longer than any EEG activity. This was true for all bird types, ages, and with all killing treatments. Similar results have been identified in previous research and confirm that the behaviours are not treatment specific (Erasmus et al., 2010a; Erasmus et al., 2010c; Gerritzen et al., 2004). Therefore neither of these behaviours are useful indicators of brain function and brain death, although their cessation is probably helpful as a very conservative measure that the bird has died and complete brain death has occurred (Dawson et al., 2009; Dawson et al., 2007; Erasmus et al., 2010a; Erasmus et al., 2010c; Gerritzen et al., 2004). The EEG data recorded in this experiment only measured the electrical potentials on the surface of the cerebral cortex, not in the brain stem; therefore the brain stem may have still been active, even though the trace had become isoelectric. This is considered to be very unlikely however, as all reflexes associated with brain stem death (e.g. rhythmic breathing) (Erasmus et al., 2010c; Widjicks, 1995) had ceased. The last behaviour to cease in the majority of birds, irrespective of killing treatment, was cloacal movement and like other convulsive behaviours it continued for longer than any reflexes or any measurable electrical potentials from the cerebral cortex. It has been suggested that the sporadic contraction and relaxation of the cloaca through spinal reflexes is not related to brain stem function and ceases once all available adenosine triphosphate (ATP) has been used within the muscle and sphincter (Solomon, 1990; Van de Sluis et al., 2009).

Time to unconsciousness was inferred in three ways: duration of reflexes (e.g. jaw tone) and latency to F50 < 12.7 Hz (maximum unconsciousness threshold) and F50 < 6.8 Hz (mean unconsciousness threshold) as a more conservative measure. The mean marker for unconsciousness matched the mean reported in Sandercock *et al* (2014) (F50 = 7 ± 2 Hz for under general anaesthesia), which established spectral variables for poultry in various chemically induced states of consciousness (e.g. awake, sedated, general anaesthesia, and isoelectric). The baseline (awake) state reported in this experiment also concurred with the mean spectral variables reported in previous research (DEFRA, 2014; Rattenborg et al., 2009; Sandercock et al., 2014). As in previous work, unconsciousness was characterised by decreasing F50 and a sharp increase in PTOT (McKeegan et al., 2013a; McKeegan et al., 2013b; Sandercock et al., 2014), which was demonstrated in all time series graphs of spectral variables for all killing methods which had EEG measures (i.e. MCD, NMCD and MARM). The use of two unconsciousness thresholds was seen as conservative and allowed a representation of the unconsciousness gradient following application of a killing method. Interestingly, the latencies for either threshold were identical within killing method for NMCD and MARM, suggesting that when the method caused sufficient physiological trauma to result in loss of consciousness, it was immediate and resulted in birds inhabiting a deep unconscious plane, where they are considered insensible (DEFRA, 2014; Sandercock et al., 2014). For MCD, the latencies for both thresholds were not identical, and potentially demonstrate birds losing consciousness more gradually. At the maximum unconsciousness threshold (F50 < 12.7 Hz) it could be suggested that birds are lightly unconscious, as it is between the gradient of sedated (drowsy, reported as F50 < 14 Hz (DEFRA, 2014; Sandercock et al., 2014)) and unconscious (surgical plane, reported as F50 < 7 Hz (DEFRA, 2014; Sandercock et al., 2014)). We cannot definitely say that birds were insensible when maximum threshold is reached, however the calculation of this threshold showed that birds did not respond to noxious stimuli (a firm comb pinch, N = 62 birds).

The MCD method was shown to result in the shortest time to F50 < 12.7 Hz (2.6 s), which from the “knock-down” spectral variables, indicated birds were unconscious and thus not sensitive to painful stimuli. The EEG analysis also demonstrated that unconsciousness was maintained until brain death, demonstrating that birds did not show any signs of recovery in cerebral electrical activity. In Sandercock et al (2014), birds were reported to have an F50 = 14 ± 4 Hz when sedated, therefore the range of < 12.7 Hz fits with descriptions of consciousness being on a gradient (Butler et al., 2005; Day et al., 1982; McIlhone et al., 2014; Sandercock et al., 2014) and that birds below the F50 < 12.7 Hz threshold can be considered in an unconsciousness state somewhere between sedated and fully unconscious (F50 < 6.8 Hz). Other research has demonstrated time to unconsciousness from EEG analysis for MCD in conscious birds, using a threshold of F50 < 7 Hz (DEFRA, 2014), and reported a mean latency of 3.6 s, which is similar to the latency reported in this study, however the slight difference could be attributed to the significantly lower sample size (N = 9).

In terms of mechanical methods, the NMCD performed the best and was not significantly different from the MCD for both the maximum (F50 < 12.7 Hz) and mean unconsciousness thresholds (F50 < 6.8 Hz), suggesting they were equivalent in terms of time to reaching unconsciousness. Continuous sampling of EEG demonstrated that birds remained unconsciousness and well within the F50 < 12.7 Hz threshold post device application and until brain death, which was reached within a significantly shorter time compared to MCD and MARM. The MARM was the least humane with the longest durations to F50 < 12.7 Hz and < 7 Hz (3.5 s for both). However, it is important to note that around treatment application (0 – 5 s) the ability to record EEG was hampered due to a high noise component in the trace, as a result of the bird’s vigorous convulsions (Delorme and Makeig, 2004; Gwin et al., 2010; Lowe et al., 2007). This caused a reduction in usable epochs around this time, and may have elongated the calculated mean for all killing treatments as a result.

All killing methods caused an initial increase in PTOT post device application, which then decreased, and continued to do so until reaching isoelectric levels. The F50 remained low post device application for all methods. This initial increase has been suggested to indicate the loss of functional cerebro-cortical activity due to synchronisation of firing neurons, increasing the overall amplitude (Bager et al., 1992; Martoft et al., 2001). This initial increase has been shown to last up to 15 s post electrical stunning due to epileptiform activity increases (Beyssen et al., 2004; Velarde et al., 2002).

There are limitations with using EEG to infer consciousness post device application in this study, as the birds were anesthetised prior to testing. However, efforts were made to reduce ongoing anaesthetic effects, by using a rapid induction method, which has shown within this study (refer to Section 5.2.3.3) and previous research to minimise long-term effects (i.e. recovery times) of the anaesthetic on the EEG pattern (Constant et al., 2005; Heinke and Koelsch, 2005). Several studies have demonstrated that anaesthesia alters EEG patterns and as a result derived variables (e.g. PTOT and F50) (Gibson et al., 2009; Gregory and Wotton, 1986). Therefore from this study it cannot be proven whether the killing treatments caused immediate loss of consciousness or not, as the sevoflurane would still be circulating within the birds' systems and potentially altering their brain state (Constant et al., 2005; Heinke and Koelsch, 2005). However, the anaesthetic effects were minimal and continuous analysis of spectral variables for all killing treatments demonstrated that they maintained unconscious states in birds until brain death.

There was no correlation between reflexes and EEG data, highlighting a concern that established consciousness indicators for poultry as well as other species may not be accurate. However, the lack of relationship appears to be always in the same fashion, with reflex durations lasting longer than the EEG unconsciousness thresholds suggest. However, previous research has shown that anaesthesia can affect reflex responses and can abolish or elongate durations of reflexes (e.g. nictitating membrane and pupillary response) (Aissou et al., 2012; Haberham et al., 1999; Haberham et al., 2000; Sandercock et al., 2014). Although, as described above, the use of rapid induction method for short-lived anaesthesia should have minimised the effect on reflex durations post treatment application. Therefore the use of the reflexes as indicators could be considered as very conservative measures of either unconsciousness or brain death, because their cessation was always longer than time to F50 < 6.8 Hz. When considering both measurements of reflex/behaviour durations and EEG analysis (e.g. latencies and time series), the NMCD device appears to be the most humane mechanical method and competes with MCD with no significant differences in terms of durations to unconsciousness. The MARM was not humane because 50% of birds were not killed and the remaining birds were potentially conscious for up to 30 s according to behavioural data.

Despite the lack of EEG data for the MZIN, the reflex data suggests it could be considered to be humane, as cranial reflex durations (pupillary, nictitating membrane, and rhythmic breathing), and jaw tone were abolished quickly post device application (all < 4.5 s). Therefore, if the reflexes are considered highly conservative compared to EEG measurements, then for this treatment, hypothetically it could be suggested to be the most humane, which would match previous findings for captive bolt devices (Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory et al., 2007; Gregory and Wotton, 1990). However, the rapid loss of reflexes must be taken within context of a relatively low kill success rate of only 75%. The rapid loss of reflexes was believed to be due to the extensive trauma caused to the head by the bolt, including direct damage to the eyes, which disrupted blood supply and injured the physiological structure (Croft, 1961; Kushner, 1998; Tidswell et al., 1987; White and Krause, 1993). Although unconsciousness could not be confirmed through EEG measures for the MZIN, the loss of all reflexes associated with brain death (and therefore also unconsciousness), suggest that birds were unconsciousness promptly after application. However, the lack of reliability of this device means that it cannot be recommended for routine use in poultry. One reflex which had previously shown promise as an indicator of loss of consciousness is jaw tone (Heard, 2000; Sandercock et al., 2014). The MZIN, NMCD and MCD all had time to loss of jaw tone means < 4.6 s and were not significantly different, but the MARM had a substantially longer duration for jaw tone, suggesting birds could be consciousness for up to 30 s after device application. However, EEG data suggested birds killed by the MARM were only conscious for roughly 3.5 s, so the relationship between jaw tone and loss of consciousness as measured by EEG remains unclear.

In terms of consistency of application and effect on bird physiology, the results must be taken in context of the kill success rate of the device. The MARM had a poor success rate, however when it was successful it did cause the intended trauma to the bird’s head, with the majority of birds receiving damage to the brain stem and cerebellum. The MARM operates in a similar fashion to pithing in rodents and cattle (Close et al., 2007), puntilla (or ‘punctilla’) in cattle, llamas and sheep (Blackmore et al., 1995; Dembo, 1894; Limon et al., 2009; Limon et al., 2010; Limon et al., 2012; Tidswell et al., 1987), and spiking in fish (Robb et al., 2000). It has been suggested that this damage renders the animal immediately unconscious through direct trauma to the brain stem itself, as well as secondary trauma through changes in intracranial pressure and reduction in blood supply

(Blackmore et al., 1995; Dembo, 1894; Krause et al., 1988; Slivka et al., 1995; White and Krause, 1993). However, like more recent research (Blackmore et al., 1995; Limon et al., 2009; Limon et al., 2010; Limon et al., 2012; Tidswell et al., 1987) and results shown here, it seems that this damage does not result in immediate loss of cranial or spinal reflexes and EEG data suggested the method was the least humane. Some studies have also noted that a singular "spike" action is not sufficient in producing fatal damage (Dembo, 1894; Limon et al., 2009), therefore multiple "spikes" may be required. This would be inhumane, as the conscious animal will receive multiple wounds, but also may also be impractical in terms of efficiency and biosecurity risks, with excessive loss of blood into the farm environment, particularly as birds that require on-farm killing may be diseased (Halvorson and Hueston, 2006; Nerlich et al., 2009).

The MZIN only killed 75% of birds, however, for all successful kills the device caused the intended effect on the anatomy, with a minimum of one, and in the majority of cases three, region(s) of the brain receiving damage, resulting in severe irreversible trauma (e.g. lacerations, contusions, axonal damage, and *contrecoup* effect, (Claassen et al., 2002; Kushner, 1998; Slivka et al., 1995; Solomon, 1990; White and Krause, 1993; Widjicks, 1995). These wounds were fatal and disrupted CNS function, shown by immediate loss of the majority of cranial reflexes. The extent of axonal damage is correlated with the amount of the brain damaged (Krause et al., 1988; White and Krause, 1993), therefore the extensive damage caused by the MZIN was likely to disrupt a large number of axons in the brain tissue, and cause unconsciousness more rapidly and maintain it for longer (Krause et al., 1988; White and Krause, 1993). Interestingly, the device caused a right-side brain damage bias, explained by the operator's left handedness. This was not ideal in terms of producing diffuse brain damage, as ideally damage should occur to both sides of the brain in order to maximize damage and disruption to CNS function. Assessment of brain damage was limited to binary (yes/no) measures per brain region, and it may be more informative in future studies to develop a grading system to score levels of damage (e.g. bruising, lacerations, etc.).

There is concern with both brain trauma methods (MARM and MZIN), that when the devices were unsuccessful they performed very poorly with less than 20% of birds receiving any brain damage, as well as up to 20% of attempts not resulting in a penetrated or fractured skull, suggesting the devices were inconsistent in their effect on the anatomy, particularly as the kill success rates were not optimal. With such low numbers of birds receiving brain damage, it is likely that the majority were not rendered unconscious (Beshkar, 2008; Krause et al., 1988; Kushner, 1998; White and Krause, 1993) and were able to perceive pain from the non-fatal injuries they had received; therefore their welfare was greatly compromised. The key issue with both MZIN and MARM were their low kill success rates. There are no published figures on acceptable failure rates for devices, but ethically it would be reasonable to suggest that a greater than 10% fail rate would be unacceptable, however in a commercial setting, the acceptable failure rate may be even lower.

MCD and NMCD were shown to be very consistent in terms of their effect on the anatomy. All successfully killed birds had their cervical vertebrae dislocated and spinal cords severed. The location of the dislocation was very consistent as well, with approximately 80% of birds receiving a C0-C1 dislocation, which focalises the trauma around the brain stem and top of the spinal cord, resulting in functional impairment and increased likelihood of neurogenic shock (Dumont et al., 2001a; Dumont et al., 2001b). The localised damage in the neural axons, results in biochemical changes (e.g. depolarization), all of which can cause a concussive effect (Brieg, 1970; Gregory et al., 1990; Shi and Pryor, 2002). The NMCD device was shown to be more consistent than MCD in causation of severing of the carotid arteries. Blood flow to the brain and brain stem is reduced by the severing or occlusion (via stretching damage) of the carotid arteries (Aslan et al., 2006; LeBlang and Nunez, 2000; Perry et al., 2012; Whittow, 2000), which results in cerebral ischemia and/or hypoxia (Gregory and Wotton, 1986; Gregory and Wotton, 1990; Solomon, 1990). The importance of severing these blood vessels has been previously highlighted (HSA, 2004), however in this study there was no effect of number of carotid arteries being severed on durations of cranial reflexes, suggesting that severing of these blood vessels is not necessary and occlusion of the vessels through stretch trauma may be sufficient. However, as a gold standard, the severing of the arteries is recommended as it results in permanent prevention of blood flow to the brain, preventing the possibility of recovery.

Additional factors which at times affected the reliability, humaneness and consistency of all killing methods were bird type, bird weight and bird age. These three factors were sometimes confounded resulting in interactions for which the cause of the effect could not be disentangled. The MARM and MZIN devices were most affected by these factors, demonstrating the limited ability of such devices to adapt to individual bird variation. Even with the MARM’s three different sized insertion cups, which were designed to compensate for this head size variation; broiler chicks and layer hens had very low kill and device success rates. In layers this is thought to be due to the excess skin on the back of the bird’s head, which allowed the spike to slip down the side of the neck and away from the target area. In the broiler chicks, the issue was primarily with the bird’s head being too small and the head slipping deeper into the cup as the spike was applied, resulting in insufficient penetration depth. The MZIN performed poorly with older and larger birds, particularly layers, probably due to the inability of the bird’s head to lie flat (dorso-ventrally) on the table surface prior to firing, due to pivoting on the keel bone and the beak tip. This was more of an issue in older birds, where the beaks were longer, but also in layer birds, which carry less muscle, making the keel bone more prominent and causing the bird to rock from side to side. The NMCD and MCD were minimally affected by these additional factors, showing their adaptability to different sizes, weights and types of birds. This seems to be mainly due to the operator’s input into the application of the killing method, both methods could be subtly adjusted immediately (e.g. creating a wider gap between fingers, more vigorous stretch for larger birds, etc.), although this was reliant on the operator’s experience and training to make an assessment of what adjustments were required.

In conclusion, the evaluation of three mechanical killing devices and the manual control method (MCD) with regard to their reliability, humaneness and consistency demonstrated that NMCD was the most successful mechanical method. The MZIN did show promise in terms of humaneness with the shortest reflex durations, although the lack of EEG data means there was no corroboration with the reflex data. This was counteracted by the success rate of the MZIN however, which was below the 90% minimum kill success rate. The MARM device performed poorly in all three areas demonstrating its lack of suitability as a humane killing method for poultry on-farm and as a result it was not taken forward in further studies to be tested on live conscious birds, based on welfare concerns. Interestingly, the manual control method (MCD) performed well as a killing method for poultry and matched the performance of NMCD. Collectively, the findings of this study provide evidence that the NMCD is a promising device for killing poultry on-farm.

6 Evaluation of reflexes and anatomical damage produced by novel and modified mechanical killing devices on conscious broilers and layers

6.1 Introduction

Assessing the effectiveness and humaneness of a killing method is achieved by determining the time to unconsciousness (insensibility) and time to brain death. The assessment of novel methods must be undertaken in a humane manner, which minimises the experience of pain and distress to the animals until the method(s) have been determined as reliable for killing birds. Chapter 5 demonstrated that the NMCD and MZIN showed adequate potential as reliable killing methods for poultry when performed on anaesthetised birds. In addition, electrocephalogram data showed that NMCD caused unconsciousness in the majority of birds by 3.1 s and that unconsciousness was maintained until the EEG trace became isoelectric, which occurred for all birds within 42 s. Disappointingly, EEG data could not be collected for MZIN, however reflex and behaviour durations indicated MZIN to cause consciousness indicating behaviours to cease immediately on device application in 97.5% of birds and brain death indicating behaviours to cease within 60 s post-application for all birds.

Several studies have identified and validated the loss of brain stem reflexes (e.g. corneal reflexes) and spinal reflexes (e.g. nociceptive withdrawal reflex) as an indicator of loss of consciousness and/or brain death in poultry (Erasmus et al., 2010a; Erasmus et al., 2010c; Sandercock et al., 2014; Verhoeven et al., 2014), as well as in several other species (Cartner et al., 2007; Croft, 1961; Hellyer et al., 1991). The lack of pupillary reflex and jaw tone have both been used as indicators of unconsciousness in poultry (Erasmus et al., 2010c; Sandercock et al., 2014), although there are reported differences between the presence/absence of certain reflexes as a result of the type of kill method, as well as induced unconsciousness via anaesthesia (Erasmus et al., 2010c; Knudsen, 2005; Sandercock et al., 2014; Verhoeven et al., 2014). For example, the pupillary reflex has been used as a method to determine complete insensibility (Anil, 1991) and brain death (Erasmus et al., 2010c; Heard, 2000), however methods which result in disruption to blood supply to the retina (e.g. slaughter methods) have been shown to affect the duration of this reflex thus not accurately indicating consciousness (Blackman et al., 1986; Erasmus et al., 2010c). Some studies have also highlighted the cessation of convulsive behaviours in poultry (e.g. wing-flapping and leg paddling) as indicators of complete brain death (Dawson et al., 2009; Dawson et al., 2007). The loss of spinal and brain stem reflexes can be attributed to physical trauma to these areas as well as the specific type and scale of trauma and therefore effect the time to brain death and loss of consciousness (Close et al., 2007; Shaw, 2002). Killing methods which cause extensive damage to the brain (including the brain stem) are likely to result in disruption of neurophysiological pathways affecting the conscious state of the bird and resulting in its death.

Although earlier work in this thesis used dead (Chapter 4) or unconscious birds (Chapter 5) in order to determine the efficacy of various methods, it was essential that further work was undertaken in live animals, to determine how effective and humane they were in a realistic context. In this study the kill efficacy of the MZIN and the NMCD was assessed in live and conscious poultry to determine the consistency of the devices in conscious birds, as well eliminating any affects that anaesthesia may have had on the reflex results of the previous trial (Chapter 5). Kill efficacy was assessed through duration of brain stem and spinal reflexes post-application and physiological damage produced through post-mortem analysis.

6.2 Materials and Methods

6.2.1 Animal housing and husbandry

This study was undertaken between March 2013 and May 2013. A total of 180 female birds were used, evenly distributed across 2 bird types and ages (broilers/layers x juveniles/adults) (Table 6.1). Birds were transported from commercial farms in two batches of 90 birds, with 30 birds (7 or 8 birds per bird type + age) assessed for each kill treatment within each batch. Upon arrival all birds were weighed and wing-tagged for identification. The birds were housed for one week prior to the experiment commencing in order to allow the birds to acclimatise to the new housing environment. All birds were housed on deep litter floor pens at lower than commercial stocking density in separate rooms per bird type and age group in order to provide recommended environmental controls for each bird strain (Aviagen, 2009; Hy-Line, 2012) as well as environmental enrichments (DEFRA, 2002a; DEFRA, 2002b). Each pen was constructed from a wooden frame with wire-grid sides and roofs (L 1.5m x W 1.0m x H 1.5m); as a result all birds had both visual and auditory contact with other birds within the same room. All birds had *ad libitum* access to feed and water, with age and bird type-specific feed (either pellets or mash) were provided. Ambient temperature was checked daily and all birds were inspected twice daily.

Table 6.1 Accommodation and bird details for each bird type and age group.

Room	No. of pens	Bird group	Age (on arrival)	Mean arrival weight (kg)	N per pen	Pen furniture
1	6	Layer pullets (Hy-Line strain)	Batch 1 – 10 wks Batch 2 – 13 wks	1.08 ± 0.02	3-4	1 feeder, 3 Spark nipple drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string
2	6	Layer hens (Hy-Line strain)	Batch 1 – 58 wks Batch 2 – 63 wks	1.79 ± 0.03	3-4	1 feeder, 3 Spark nipple drinkers, 1 wooden perch, 1 nest box, 2 x suspended blue string
3	1	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 bell drinker, 4 x suspended shiny objects
4	10	Broiler – slaughter age (Ross 308 strain)	Batch 1 – 5 wks Batch 2 – 5 wks	2.17 ± 0.06	2-3	1 feeder, 3 Spark nipple drinkers, 2 x suspended shiny objects

6.2.2 Study design

Two novel or modified mechanical poultry killing devices, MZIN and a NMCD were assessed for their kill efficacy in comparison with each other and a control (MCD). Details of the device designs are provided in Chapter 3.3.1 and 3.3.4. The MCD method was performed in the standard manner, described in Chapter 3.4.

The kill treatments were tested on all 180 birds across the two bird types and ages resulting in 15 birds per bird type + age for each kill treatment (Table 6.2). Across the two batches a Latin-Square design was used to systematically randomise kill treatment, bird type + age and kill order. Kill treatment was allocated to individual birds so as not to confound kill treatment with pen groupings. Birds were killed over a 5 day period for each batch, with 18 birds killed per day. All kill treatments were applied by one trained and experienced operator (the author). Elastic bandage (Vetrap^{TM}) was wrapped around the bird's body and over the wings immediately prior to killing in order to minimise excessive convulsive movement from the wings, which could hamper the visibility of behavioural measures.

Table 6.2 Total number of birds per killing method and bird type and age group.

Bird group	**MZIN**	**NMCD**	**MCD**	**Total**
Layer pullets	15	15	15	45
Layer hens	15	15	15	45
Broiler chicks	15	15	15	45
Broiler – slaughter age	15	15	15	45
Total	60	60	60	180

The tests were digitally video recorded by two cameras (Low-lux B/W waterproof cameras: SK-2020XC/SO, RF Concepts Ltd, Belfast, Ireland and Geovision GV-DVR, ezCCTV Ltd, Herts, UK) from the point of killing method application through to 30 s after all behaviours and reflexes had ceased. The video footage from both cameras (camera 1was aimed at the bird's body; camera 2 was aimed and zoomed in on the bird's head) allowed back-up observations to be performed, if live observations of behaviours and reflexes were missed. The efficacy of the devices was determined in three ways: (1) derived kill success and device success measures; (2) durations of reflexes and behaviours post treatment application; and (3) post mortem analysis.

6.2.2.3 Kill success and Device success

Similar to the previous experiment, killing performance was scored in two ways: kill success and device success, with details described in Chapter 5.2.2.1.

6.2.2.1 Behavioural and reflex measures

Behavioural observations were performed following the method described in Chapter 5.2.2.4, with reflexes/behaviours assessed as present or absent in 15 s intervals post killing method application, until a consecutive 30 s of absence of all behaviours and reflexes was observed.

6.2.2.2 Post-mortem evaluation

Post-mortem assessment was performed on every bird immediately after the bird was confirmed dead. Specific post-mortem measures were taken for certain killing methods as their target areas were different. For all killing methods binary yes/no measures were recorded for whether the skin was broken, signs of external blood loss and subcutaneous hematoma. For MZIN, seven specific measures were recorded: skull penetration location (as described previously in Chapter 5 - Figure 5.2); a four point grading (Table 6.3) of damage to the left forebrain, right forebrain, cerebellum, midbrain and brainstem; and a binary measure (yes/no) of the presence of an internal brain cavity hematoma. Post mortem measures for the NMCD and MCD killing methods are described in Section 5.2.2.5.

Table 6.3 Grading system for categorising levels of damage to individual areas of the brain.

Damage grading	Description
None	No damage to the specific region of the brain, no visual bruising or physical damage.
Low	Region of brain is physically intact; however there is visual bruising and pooling of blood in the surrounding area.
Mid	Region of brain shows visual 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 visual bruising and bleeding in the surrounding area.

6.2.3 Statistical Analysis

All data were summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat (14^{th} Edition). Summary graphs and statistics were produced at the bird level. Statistical comparisons for kill success and device success were conducted via Generalised Linear Mixed Models (GLMMs), using logit link function and binomial distribution. Statistical significance was defined by a threshold of 5% level and based on F tests. For all models the random effects included the batch, date and the bird ID. All fixed effects were treated as factors and classed as categorical classifications.

6.2.3.1 Kill and Device Success

Described in Chapter 5.2.3.1.

6.2.3.2 Behavioural and reflex data analysis

Described in Chapter 5.2.3.4.

6.2.3.3 Post-mortem analysis

Described in Chapter 5.2.3.5. Additional GLMMs using logit link function and binomial distributed were conducted for the categorised measures of brain damage grade per brain area (refer to Table 6.3). Fixed effects included were killing method, bird type, bird age, bird weight, skull penetration location, and their interactions.

6.3 Results

Body weight and bird age ranges did not overlap across the different bird groups. Mean weights (± SE) at the time of kill were: broiler chick 0.71 ± 0.02 kg; broiler (slaughter age) 2.17 ± 0.06 kg; layer pullet 1.08 ± 0.02 kg; and layer hen 1.79 ± 0.03 kg. Mean bird ages (± SE) were: broiler chick 17.0 ± 0.4 days; broiler 35.0 ± 0.0 days; layer pullet 12.9 ± 0.2 wks; and layer hen 62.1 ± 0.3 wks.

6.3.1 Kill success

Both the NMCD and MCD methods had 100% kill success across all birds. A total of 17 birds were not killed in the first attempt with MZIN and had to be emergency killed. Kill success was significantly affected by kill treatment ($F_{2,167}$ = 19.96, P < 0.001), with NMCD and MCD achieving 100.0% kill success rate and the MZIN achieving 71.7% (Figure 6.1). Kill order had no effect on kill success ($F_{1,167}$ = 1.14, P = 0.289) and neither did its interaction with killing method ($F_{1,167}$ = 0.94, P = 0.320). Bird type had no significant effect on kill success, although there was a significant interaction between kill treatment and bird type for kill success ($F_{2,167}$ = 3.29, P = 0.04), with MZIN layers being less successful than MZIN broilers, and all other killing method/bird type interactions being more successful in killing than either MZIN/bird type interactions. Bird age, kill weight and all other interactions had no effect on kill success.

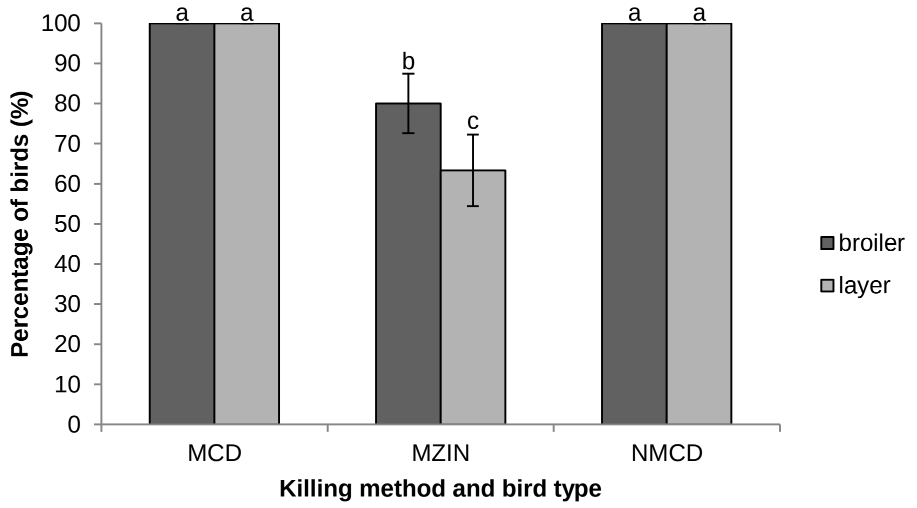


Figure 6.1 Mean (± SE) kill success rates (%) across the three killing methods and bird type (broiler/layer). No common superscript indicates that there is a significant difference between the groups.

Device success ($F_{2,167}$ = 7.33, P < 0.001) was significantly higher in the MZIN (70.0%) compared to the MCD (26.7%) and NMCD (41.7%). Kill order ($F_{1,167}$ = 0.08, P = 0.813) had no effect on device success and neither did its interaction with killing method ($F_{1,167}$ = 0.29, P = 0.729). Bird type had a significant effect on device success ($F_{1,167}$ = 9.55, P = 0.002), with the device more likely to succeed in the intended way with broilers than layers. There was also an interaction between bird type and killing method ($F_{1,167}$ = 4.23, P = 0.036) with device success higher in MZIN applied to broilers (Figure 6.2). Bird age, kill weight and all other interactions had no effect on device success.

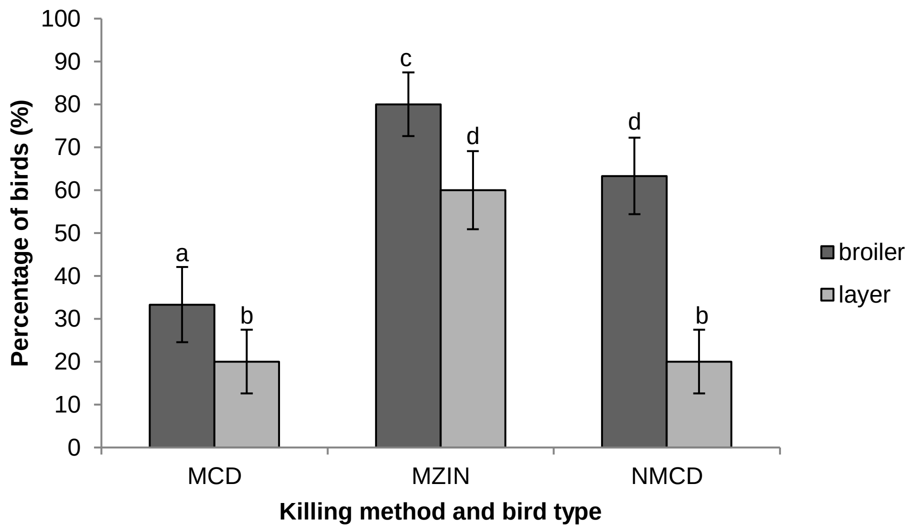
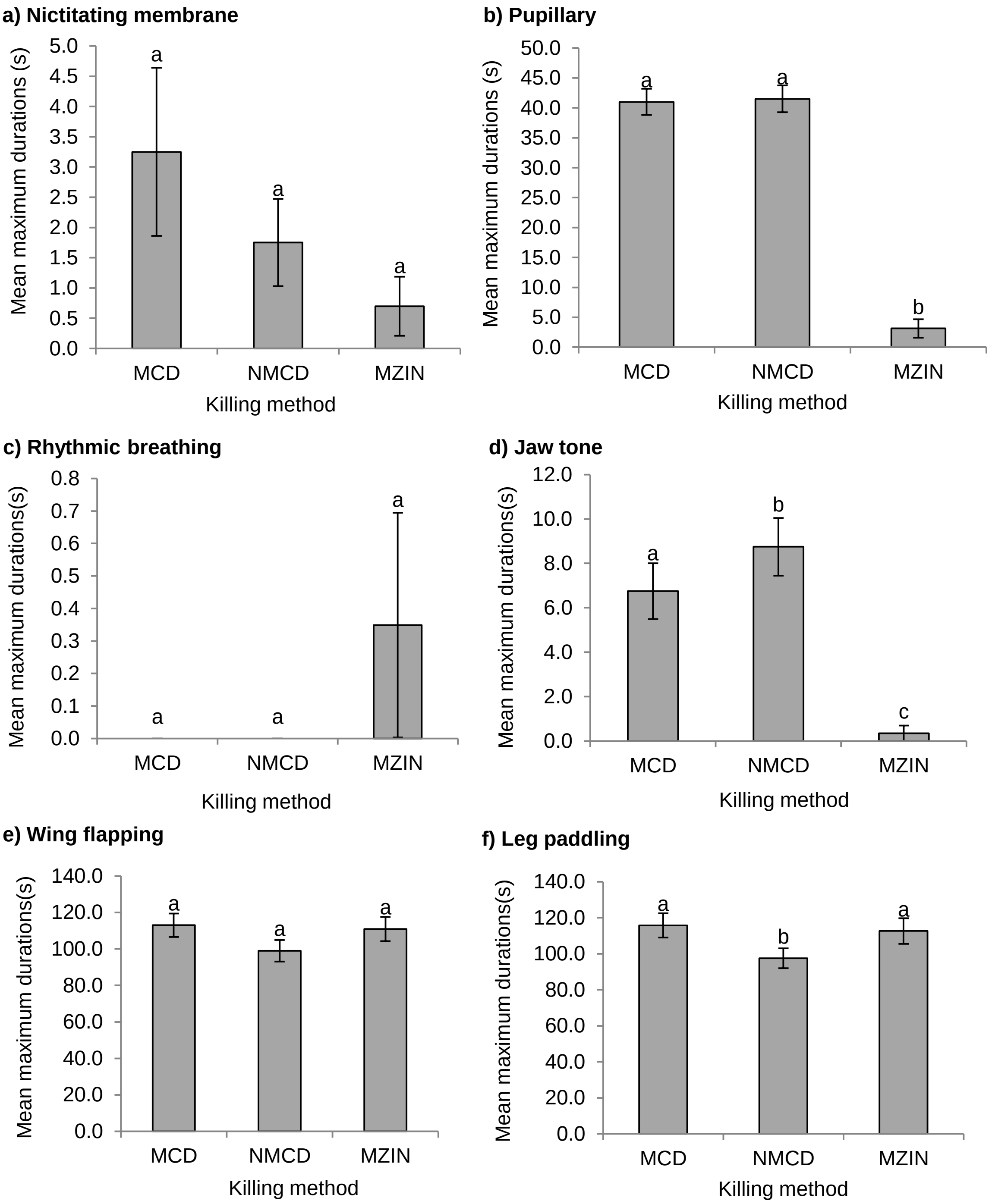


Figure 6.2 Mean (± SE) device success rates (%) across the three killing methods and bird type (broiler/layer). No common superscript indicates that there is a significant difference between the groups.

6.3.2 Behavioural observations

Mean maximum durations for reflexes and behaviours are shown in Figure 6.3. Killing method had no effect on nictitating membrane (Figure 6.3a, $F_{2,150} = 1.67$, $P = 0.191$), rhythmic breathing (Figure 6.3c, $F_{2,150} = 1.46$, $P = 0.235$) or wing flapping (Figure 6.3e, $F_{2,150} = 2.05$, $P = 0.132$). However, killing method did have an effect on pupillary reflex (Figure 6.3b, $F_{2,150} = 101.66$, $P < 0.001$), jaw tone (Figure 6.3d, $F_{2,150} = 13.34$, $P < 0.001$), leg paddling (Figure 6.3f, $F_{2,150} = 3.18$, $P = 0.044$) and cloacal movement (Figure 6.3g, $F_{2,150} = 3.75$, $P = 0.026$).



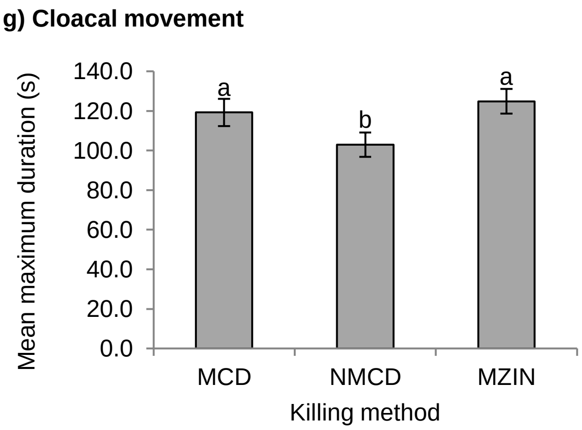


Figure 6.3 Mean maximum durations (s) across the three killing treatments for all observed behaviours and reflexes: a) nictitating membrane; b) pupillary; c) rhythmic breathing; d) jaw tone; e) wing flapping; f) leg paddling; g) cloacal movement. Please note that the y axes scales vary. No common superscript indicates that there is a significant difference between the groups.

Device success had a significant effect on rhythmic breathing durations ($F_{1,150}$ = 6.10, P = 0.015) and a tendency to affect nictitating membrane maximum durations ($F_{1,150}$ = 3.86, P = 0.051), with both having shorter maximum durations in birds which achieved device success (Figure 6.4). Device success had no effect on all other reflexes or behaviours (Table 6.4).

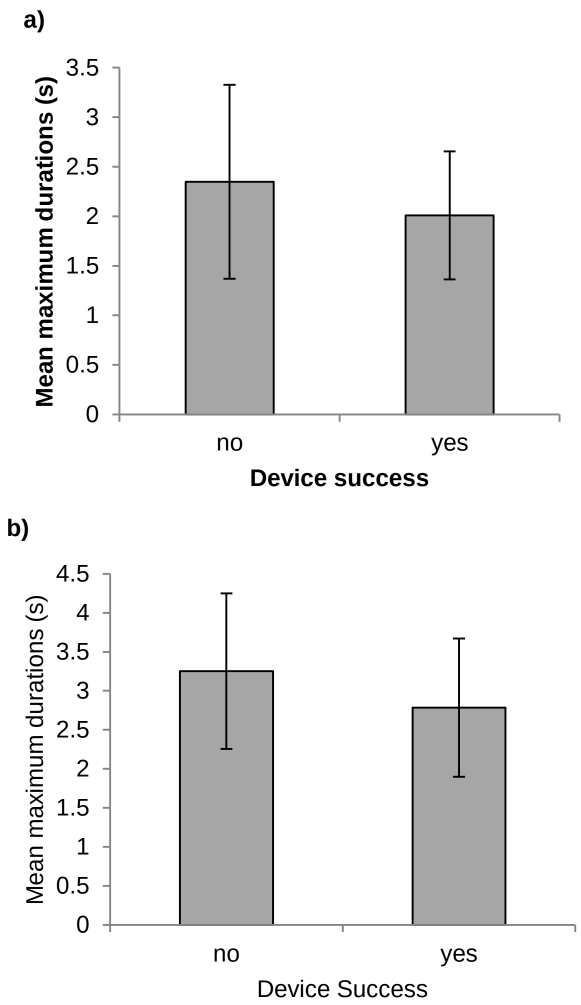


Figure 6.4 Effect of device success on the mean maximum durations (±SE) for: (a) rhythmic breathing; and (b) nictitating membrane.

Table 6.4 Non-significant effects of device success on reflexes and behaviours maximum durations, listing mean (±SE) and GLMM output. All degrees of freedom = 1 (N = 163).

Reflex/behaviour	*F statistic*	*P*	Device Success No Mean	No SE	Yes Mean	Yes SE
Jaw tone	1.28	0.260	7.41	1.04	6.186	0.977
Pupillary	1.77	0.186	34.88	2.40	26.29	2.23
Leg paddling	0.33	0.565	103.01	6.09	95.72	7.40
Wing flapping	0.93	0.337	102.65	8.07	94.48	6.18
Cloacal movement	0.11	0.744	104.38	5.53	103.71	6.67

Nictitating membrane maximum durations were affected by bird weight ($F_{1,150}$ = 5.09, P = 0.025) with heavier birds (mean = 3.33 ± 0.87 s) having higher maximum durations then lighter birds (mean = 2.23 ± 0.95 s), but there was no significant interaction between bird weight and killing method ($F_{2,150}$ = 0.61, P = 0.587). Neither bird type ($F_{1,150}$ = 0.09, P = 0.771) nor its interaction with killing method ($F_{2,150}$ = 0.41, P = 0.664) had an effect on nictitating membrane maximum durations. Bird age had no effect on maximum nictitating membrane durations ($F_{1,150}$ = 0.02, P = 0.951), however its interaction with killing method did ($F_{2,150}$ = 5.19, P = 0.007) (Figure 6.5).

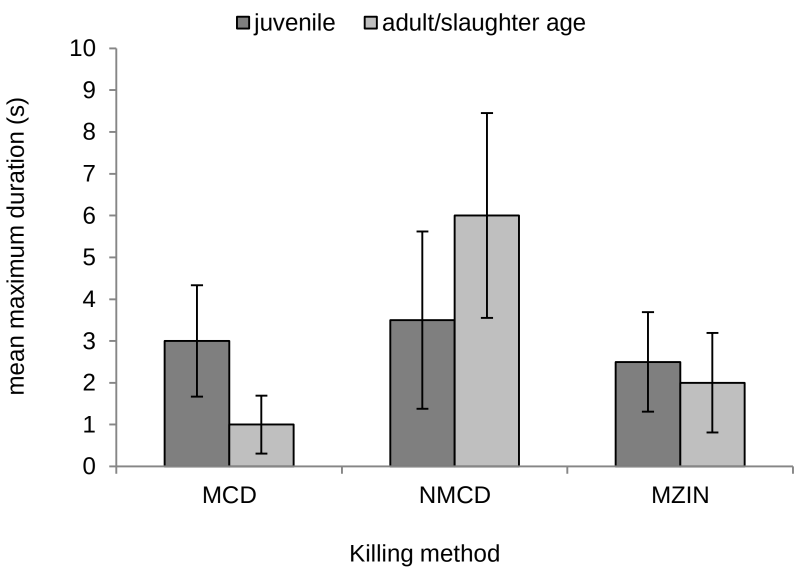


Figure 6.5 Effect of the interaction between grouped bird age and killing method on the duration of nictitating membrane reflex.

The duration of the pupillary reflex was affect by bird type ($F_{1,150}$ = 4.82, P = 0.030), and the interaction with killing method ($F_{2,150}$ = 3.58, P = 0.030), with layer type birds generally showing higher maximum pupillary durations (33.5 ± 2.5 s) compared to broilers (27.0 ± 2.2 s), apart for MCD (Figure 6.6). Bird age ($F_{1,150}$ = 6.10, P = 0.015) also had an effect on the maximum duration of the pupillary reflex with older grouped birds showing higher maximum pupillary durations (40.2 ± 5.7 s) compared to younger grouped birds (22.5 ± 3.8 s). Bird weight ($F_{1,150}$ = 0.30, P = 0.0.582) and its interaction with killing method ($F_{2,150}$ = 0.31, P = 0.0.735) or bird age ($F_{2,150}$ = 1.76, P = 0.176) had no effect on maximum pupillary durations.

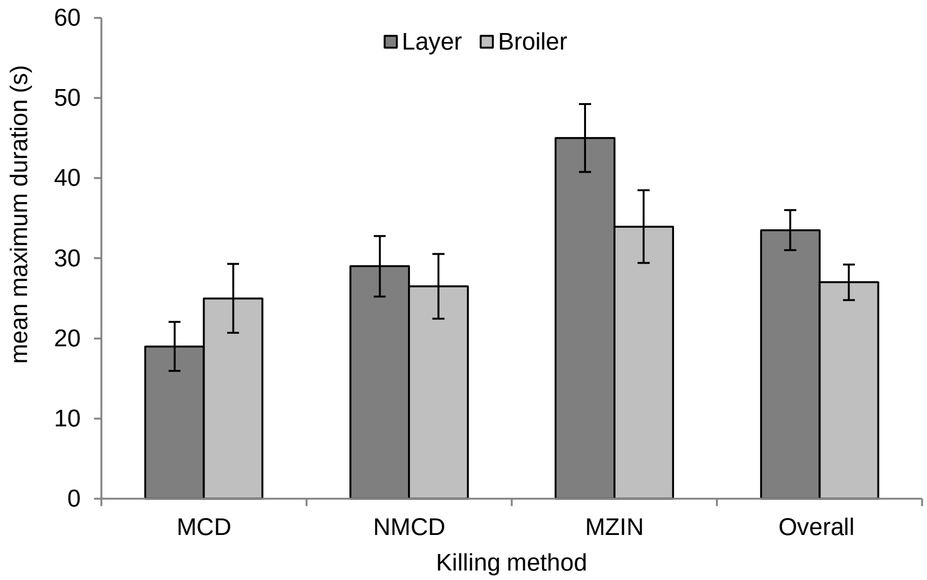


Figure 6.6 Effect of the interaction between bird type and killing method on mean (±SE) maximum pupillary durations.

The only device associated with rhythmic breathing post-application was the MZIN, but this occurred in just one case of a laying hen and was only present for the first 15 s interval. Other than device success (Figure 6.4a), no other factors or interactions had an effect on rhythmic breathing (Table 6.5).

Table 6.5 GLMM analysis output of the minimum model for the maximum duration of rhythmic breathing (N = 163).

Factor	**df**	***F* statistic**	***P value***
Device	2	1.46	0.235
Bird type	1	1.31	0.254
Bird age	1	1.48	0.225
Bird weight	1	0.06	0.810
Device success	1	6.10	0.015
Killing method. Bird type	2	0.09	0.910
Killing method. Bird age	2	1.52	0.222
Killing method. Bird weight	2	0.03	0.970

The maximum duration for jaw tone was significantly affected by killing method (Table 6.6), with MZIN having the shortest duration in comparison to NMCD and MCD (Figure 6.3); however, device success had no effect. Bird type, bird age and bird weight did not have an effect on jaw tone maximum durations. However, the interaction between killing method and bird type was shown to have an effect: broilers had shorter jaw tone durations with MZIN and NMCD (MZIN = 8.75 ± 0.21 s; NMCD = 3.50 ± 1.38 s) compared to layers (MZIN = 9.47 ± 0.46 s; NMCD = 5.00 ± 1.50 s), but MCD showed no significant differences between layers and broilers (broiler = 6.50 ± 1.71 s; layer = 7.00 ± 1.87 s). The interaction between killing method and bird age had no effect.

Table 6.6 Results of the minimal GLMM model analysis for maximum durations (s) for jaw tone, leg paddling, wing flapping and cloacal movement post kill treatment application (N = 163).

		Wing flapping		Leg paddling		Cloacal movement		Jaw tone	
Fixed Effects	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Killing method	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
Killing method. bird type	2,150	1.16	0.315	0.57	0.567	1.65	0.196	3.73	0.026
Killing method. bird age	2,150	2.23	0.111	2.23	0.111	0.63	0.533	1.62	0.180

Note: Significant P values are underlined.

Leg paddling, wing flapping and cloacal movement were affected by killing treatment (Figure 6.3) as well as bird type and bird age (Table 6.6). However no interactions between them were significant. Across all behaviours, layers and older birds had significant longer maximum durations compared to broilers and younger birds (Table 6.7). Wing flapping and leg paddling were not affected by any other factors. However, cloacal movement was affected by bird weight, with lighter birds showing elongated maximum cloacal movement durations compared to heavier birds (Figure 6.7).

Table 6.7 Mean and SE (±) of maximum durations of wing flapping, leg paddling and cloacal movement in relation to bird type and age groups.

	Wing flapping		Leg paddling		Cloacal movement	
Factor	**Mean**	**SE (±)**	**Mean**	**SE (±)**	**Mean**	**SE (±)**
Broiler	87.68	4.39	89.46	4.84	101.07	4.27
Layer	128.16	5.00	128.15	4.94	129.30	6.04
Juvenile	81.98	5.96	84.77	7.11	97.33	5.43
Adult/slaughter age	116.71	6.86	115.24	6.66	108.29	8.32

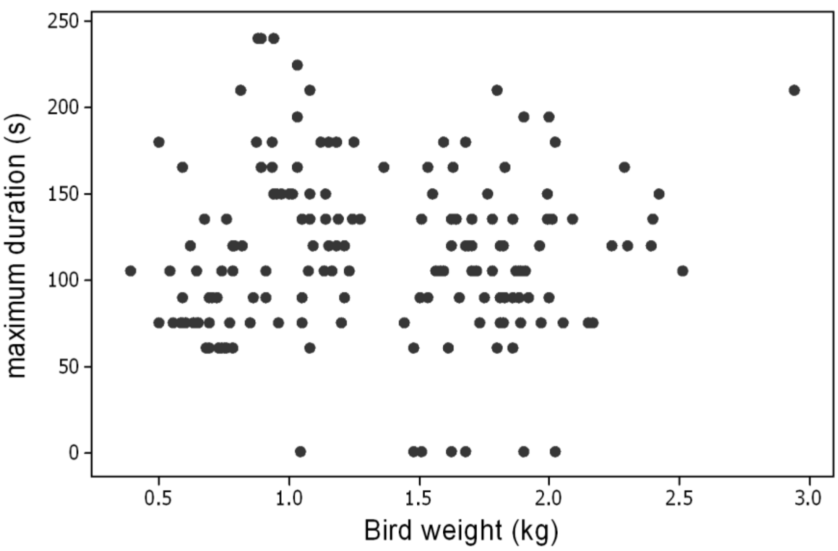


Figure 6.7 Effect of bird weight (kg) on the observed maximum durations (s) for cloacal movement.

Interestingly, the percentage of birds that showed the reflexes and behaviours at all varied by kill method, although the MCD and NMCD were similar (Table 6.8). For the cranial reflexes (nictitating membrane and pupillary) both the MCD and NMCD had higher percentages of birds displaying these reflexes post kill compared to MZIN. However, the MZIN was the only kill treatment in which one bird showed rhythmic breathing following a successful kill. In all kill treatments the majority of birds displayed convulsive behaviours post-application (e.g. wing flapping and leg paddling) and the last behaviour to cease was cloacal movement (Figure 6.3). In a small number of birds cloacal movement was not observed however this was due to the bird defecating and the movement being hidden as a result.

Table 6.8 Percentages (%) of birds which displayed reflexes and behaviours for each kill treatment. Percentages calculated from total birds per kill treatment that were successfully killed.

Reflex/ behaviour	MCD (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

6.3.3 Post-mortem evaluations

6.3.3.1 Cervical dislocation killing methods

For successfully killed birds, post-mortem results showed that both the NMCD and MCD (which both had 100% kill success rates) caused a subcutaneous hematoma in the neck, damage to the neck muscle, cervical dislocation and spinal cord severance in 100% of birds. A small proportion of birds showed minor tears to the skin (MCD – 6.7%; NMCD – 8.3%), with an even small proportion exhibiting external blood loss from the wounds (both at 5%). There were no significant differences between these two killing methods on skin tears or external blood loss. There was no difference between the NMCD and MCD for where the dislocation occurred ($F_{1,103}$ = 0.79, P = 0376), although the MCD had the lowest break at C3-C4 (Figure 6.8). Bird type and bird age had an effect on dislocation level, with layers (mean = 1.47 ± 0.10) and older birds (mean = 1.83 ± 0.16) more likely to result in lower dislocations (≥ C1-C2) compared to broilers (mean = 1.00 ± 0.00) and younger birds (mean = 1.00 ± 0.00) (Table 6.9). Interestingly, dislocation level had no effect on the maximum durations for all reflexes and behaviours. However, there was a significant interaction between dislocation level and killing method for maximum nictitating membrane duration, which demonstrated for higher dislocation levels, MCD was associated with shorter maximum nictitating membrane durations compared to NMCD, however for dislocation levels greater the C2-C3 there was no significant difference between killing methods, although the N understandably varied across dislocation levels (Figure 6.9).

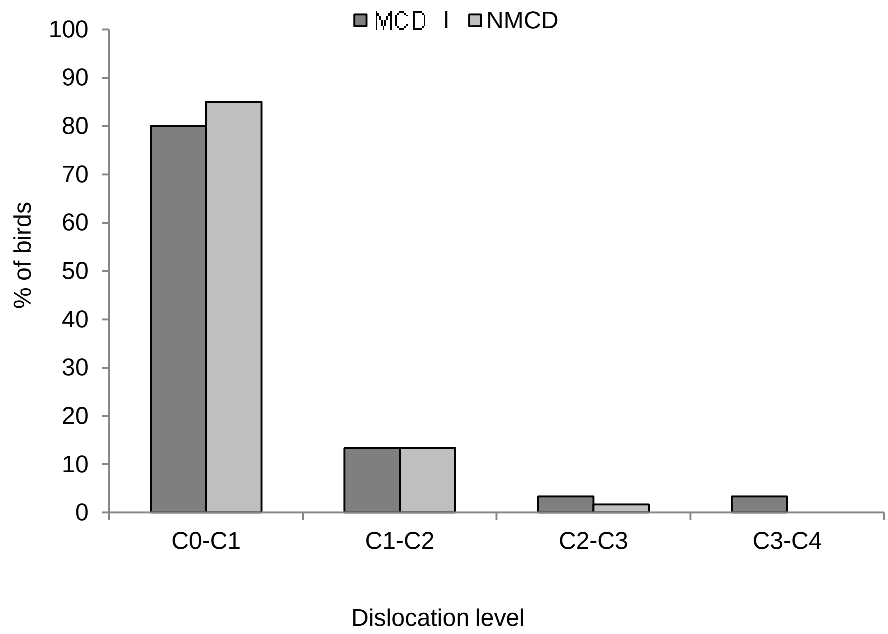


Figure 6.8 Percentage (%) distribution of birds across the range of dislocation levels produced by the MCD and NMCD killing methods.

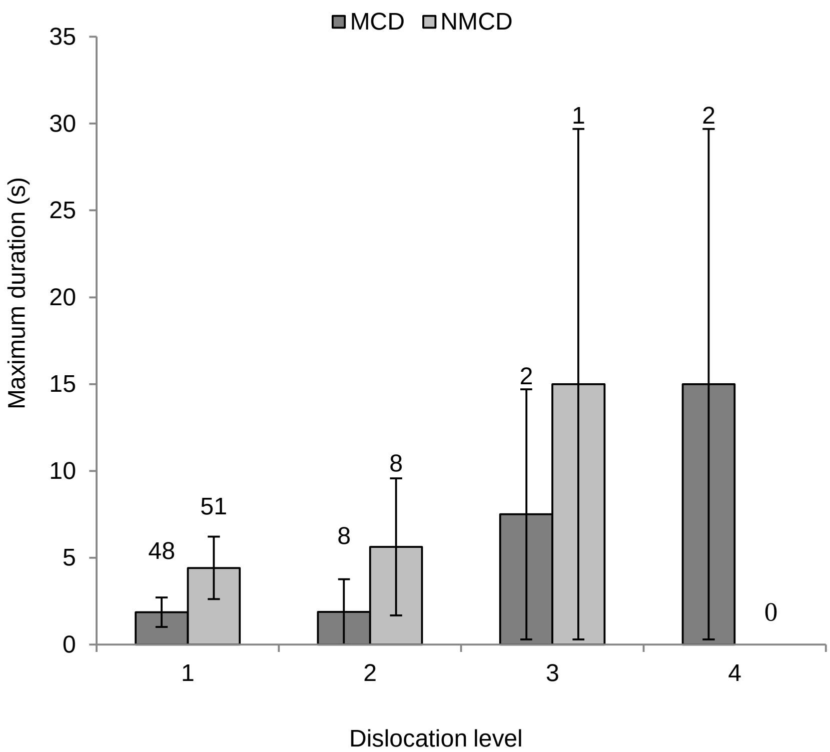


Figure 6.9 Effect of the interaction between killing method and dislocation level on the maximum duration of nictitating membrane (s). Dislocation point means were calculated by converting vertebral levels to a numerical category (e.g. C0-C1 = 1; C1-C2 = 2; C2-C3 = 3; and C3-C4 = 4). N for each killing method at each dislocation level is labelled above each bar graph.

Table 6.9 Output (df, *F* statistic and *P value*) of minimal GLMM models for post-mortem variables dislocation level, neck gap size, vertebral damage and number of carotid arteries severed.

		Dislocation level		**Neck gap size**		**Vertebral damage**		**Number of carotid arteries severed**	
Factor	**df**	***F* statistic**	***P value***	***F* statistic**	***P value***	***F* statistic**	***P value***	***F* statistic**	***P value***
Killing method	1	0.79	0.376	7.65	<u>0.007</u>	1.94	0.167	4.85	<u>0.030</u>
Bird type	1	32.00	<u><0.001</u>	0.28	0.595	2.02	0.158	2.29	0.133
Kill age	1	32.14	<u><0.001</u>	0.52	0.474	4.08	<u>0.046</u>	0.02	0.876
Kill weight	1	0.05	0.828	25.39	<u><0.001</u>	0.25	0.617	1.54	0.218
Number of carotid arteries severed	2	0.91	0.405	34.32	<u><0.001</u>	0.97	0.326	-	-
Dislocation level	3	-	-	0.11	0.746	0.82	0.487	1.39	0.250
Neck gap size	1	0.10	0.749	-	-	0.40	0.678	22.05	<u><0.001</u>
Killing method. Bird type	1	0.13	0.723	0.44	0.510	0.28	0.598	1.26	0.053
Killing method. Bird age	1	0.91	0.344	1.61	0.207	4.43	<u>0.038</u>	0.41	0.860
Killing method. Bird weight	1	0.24	0.628	0.26	0.611	0.02	0.881	0.37	0.319
Killing method. Neck gap size	1	0.29	0.592	-	-	0.46	0.631	0.20	0.789
Killing method. Number of carotid arteries severed	2	0.85	0.429	2.73	0.101	0.53	0.469	-	-
Killing method. Dislocation level	3	-	-	0.40	0.530	0.47	0.629	1.76	0.177

Note: Significant P values are underlined.

The NMCD did not cause vertebrae damage in any birds as a result of the dislocation, but the MCD caused damage in 3.3% of birds; however this difference was not significant (Table 6.9). There was a significant interaction between killing method and bird age with the only two birds receiving damage to their vertebra being 62 week old birds (layer hens), both killed with the MCD method. All other factors had no effect on vertebrae damage (e.g. bird type, bird age, bird weight, dislocation level, and all interactions) (Table 6.9).

The gap size between the two points of cervical dislocation was significantly affected by killing method and bird weight (Table 6.9). The NMCD method was more likely to result in a larger gap size compared to the MCD, with means of 6.29 ± 0.27 cm and 5.47 ± 0.21 cm respectively. Heavier birds (mean = 6.80 ± 0.27 cm) were more likely to have large neck gap sizes compared to lighter birds (mean = 5.03 ± 0.28 cm), although this is confounded by smaller birds having a smaller neck stretch capacity compared to a larger/heavier bird. Bird type, bird age, dislocation level and all interactions did not have a significant effect on gap size. No bird was fully decapitated during the experiment and maximum neck gap sizes for each killing method were 9.0 cm for MCD and 10.0 cm for NMCD.

The number of carotid arteries severed was significantly affected by killing method (Table 6.9), with NMCD more likely to sever one or more carotid arteries compared to MCD (means: NMCD = 1.22 ± 0.11; MCD = 0.90 ± 0.11). NMCD resulted in 71.7% of birds having one or more carotid arteries severed, compared to MCD where only 58.3% of birds had one or more carotid arteries severed. The number of carotid arteries severed was also significantly affected by neck gap size, with larger neck gap sizes being positively associated with more carotid arteries being severed (Figure 6.10). The interaction between killing method and bird type had a tendency to affect the number of carotid arteries severed, with broilers killed by NMCD more likely to have one or more carotid arteries severed compared to both bird types killed by the MCD, as well as layers killed by NMCD (Figure 6.11). Bird type, age, weight, dislocation level and all remaining interactions did not have a significant effect on number of carotid arteries severed (Table 6.9).

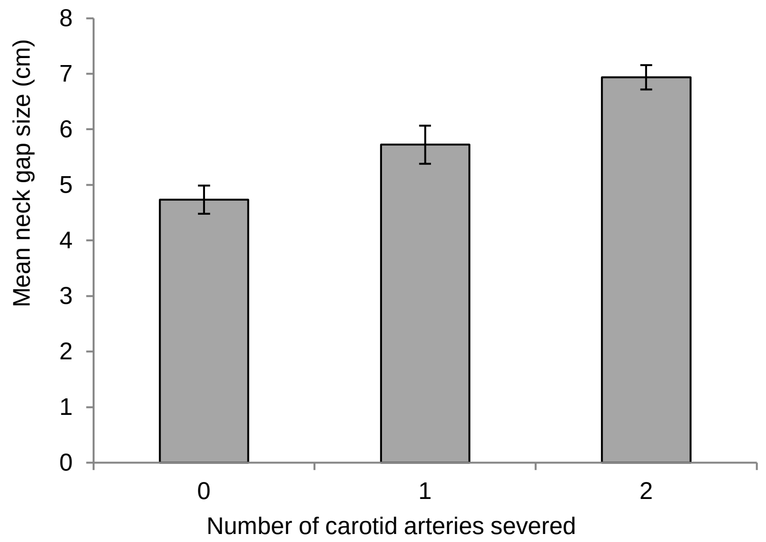


Figure 6.10 Association of neck gap size and the number of carotid arteries severed as a result of a cervical dislocation killing method.

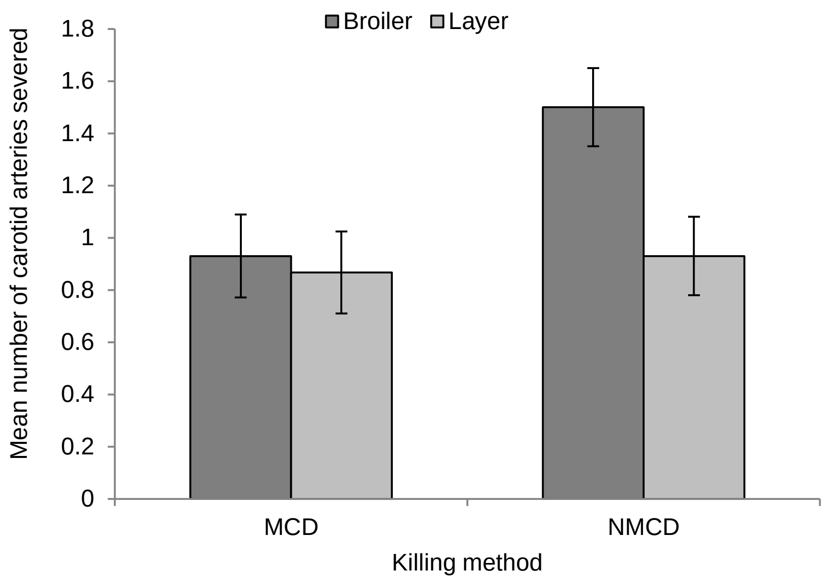


Figure 6.11 Mean (±SE) number of carotid arteries severed associated with the interaction between killing method and bird type.

The number of carotid arteries severed did not have an effect on any maximum durations for reflexes and behaviours measured, apart from having a tendency to affect jaw tone ($F_{2,102}$ = 2.53, P = 0.095). Maximum jaw tone durations were not affected by severing one or two carotid arteries for combined killing methods and NMCD individually, but when zero carotid arteries were severed, there was a significant increase in maximum jaw tone duration for the combined methods and for NMCD individually (Figure 6.12). For MCD individually, there were no differences.

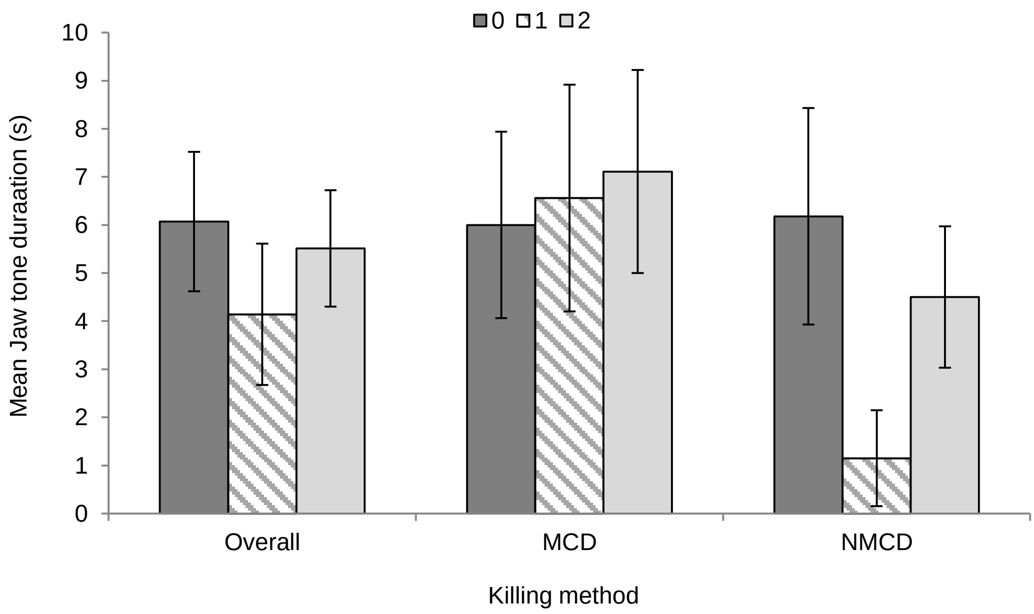


Figure 6.12 Mean (±SE) jaw tone durations (s) in relation to the number of carotid arteries (0, 1 or 2) severed for the combined cervical dislocating methods, MCD and NMCD.

6.3.3.2 Captive bolt killing method (MZIN)

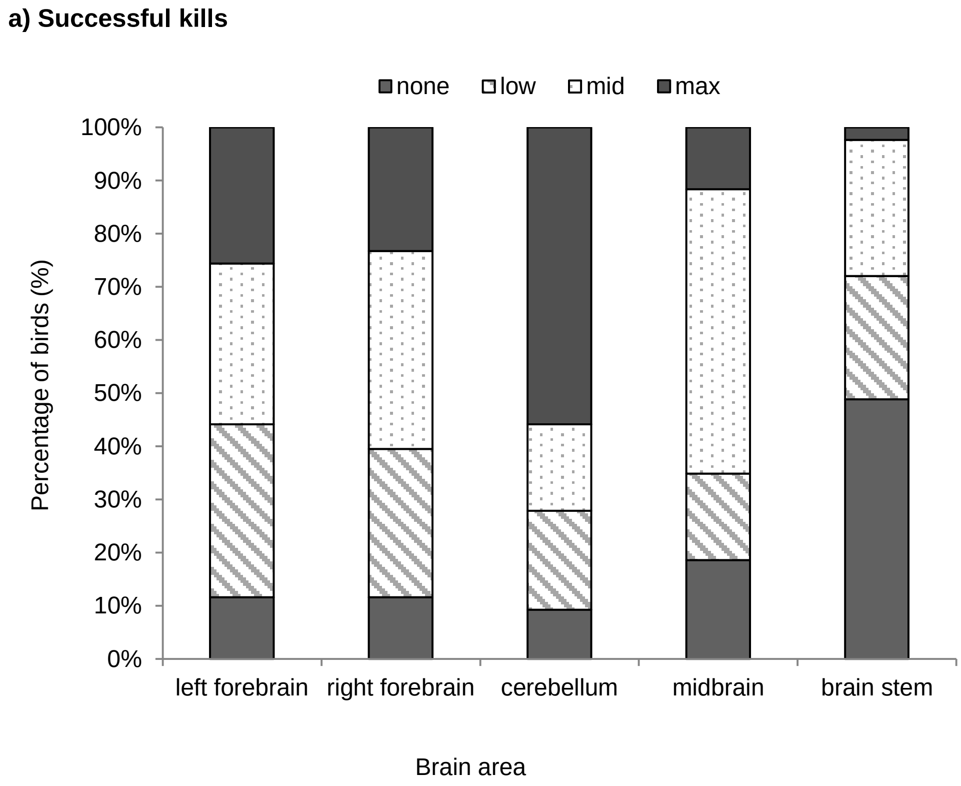
The MZIN had a 71.7% kill success rate and caused trauma to the head of the bird rather than the neck, therefore comparisons of post-mortem trauma with NMCD and the MCD are not relevant. Comparisons of physiological trauma caused by successful and unsuccessful kills for the MZIN showed significant differences across several factors (Table 6.10). Kill success did not have significant effect on skin broken, external bleeding and subcutaneous hematomas, with over 85% of birds displaying these irrespective of kill success. There was a significant effect of kill success on skull damage but there was no significant effect in terms of where the skull was penetrated by the bolt ($F_{1,43}$ = 0.19, P = 0.664) (refer to Chapter 5.2.2.5 - Figure 5.2). Device success had a significant effect on where the skull was penetrated, with birds which achieved device success being more likely to have their skulls penetrated at CB and CM, with 79.1% of all MZIN treated birds having damage in these two areas of the skull. The bird type, age, weight and all interactions did not have a significant effect on the skull penetration area.

Table 6.10 Comparison of the percentage of birds which displayed individual post-mortem measures, dependent on kill success (YES/N0) when killed with MZIN. Significant *P* values (*P* < 0.05) are underlined.

	Percentage of birds observed (%)		df	*F* statistic	*P* value
Post-mortem measure	**Kill Success 'Y'**	**Kill Success 'N'**			
Skin broken	95.4	88.2	1	0.21	0.754
External bleeding	95.4	88.2	1	0.22	0.754
Subcutaneous hematoma *	100.0	100.0	1	-	-
Skull damage	100.0	58.8	1	3.21	0.024
Internal brain cavity hematoma	100.0	64.7	1	5.57	0.018
Left forebrain damage	88.4	11.8	1	28.23	<0.001
Right forebrain damage	88.4	23.5	1	12.35	<0.001
Cerebellum damage	90.7	41.2	1	5.10	0.028
Midbrain damage	81.4	5.9	1	20.44	<0.001
Brain stem damage	51.2	0.0	1	11.63	<0.001

** GLMM analysis not run due to no variation within variable.*

Irrespective of kill success, more than 50% of the birds sustained an internal brain cavity hematoma. Kill success had a significant effect on the presence of an internal brain cavity hematoma with MZIN, with successfully killed birds more likely to have bleeding within the skull. Device success, bird type and all interactions did not have a significant effect. Bird age ($F_{1,43}$ = 16.47, P <0.001) and weight ($F_{1,43}$ = 19.09, P < 0.001) had a significant effect, with heavier and younger birds more likely to have internal brain cavity hematomas (93.3% and 90.0% respectively), compared to lighter (86.7%) and older birds (76.7%).



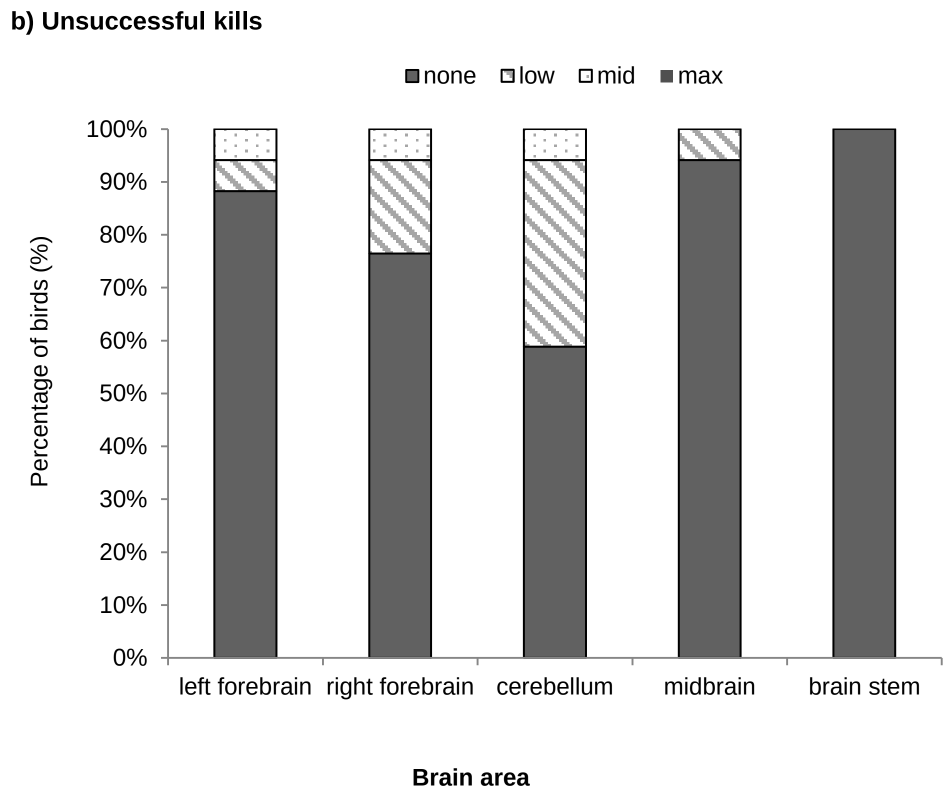


Figure 6.13 Comparison of brain damage ranges for successful and unsuccessful kills by the MZIN. Refer to Section 6.2.2.2 for defined damage grading.

More than 80% of birds successfully killed by the MZIN had damage to all main areas of the brain, excluding the brain stem, to which just over 50% of birds received damage. The grade of damage to each brain region dependent on kill success is demonstrated in Figure 6.13. Kill success had a significant effect on whether or not a brain region was damaged and the grade of the damage (Table 6.10). Forebrain hemispheres, the cerebellum, and brain stem had no other significant factors influencing whether they were damaged or not (e.g. bird type, age, weight, interactions - Table 6.11). Bird type ($F_{1,43}$ = 6.03, P = 0.014) was the only factor that had a significant effect on damage to the midbrain, with broilers (mean = 1.37 ± 0.18) more likely to sustain damage than layers (mean = 0.93 ± 0.19). Damage grade means were calculated by converting grade levels to a numerical category (e.g. none = 0; low = 1; mid = 2; and max = 3). Only in successfully killed birds did the highest grade of damage occur (max), with the cerebellum sustaining the highest proportion of damage. In unsuccessful kills, less than 45% of birds sustained brain damage and the brain stem was never damaged.

Table 6.11 GLMM output (F statistic and P value) for all variables for brain area damage (all df = 1). Significant *P* values are underlined.

	Brain stem		Cerebellum		Left forebrain		Right forebrain		Midbrain	
Factor	***F* statistic**	***P* value**	***F* statistic**	***P* value**	***F* statistic**	***P* value**	***F* statistic**	***P* value**	***F* statistic**	***P* value**
device success	0.98	0.323	0.61	0.439	3.00	0.090	2.39	0.128	2.31	0.129
Bird type	0.89	0.345	0.09	0.760	0.87	0.355	0.24	0.623	6.03	<u>0.014</u>
Bird age	0.03	0.868	2.57	0.115	0.58	0.452	3.96	0.052	2.54	0.110
Bird weight	0.00	0.990	0.13	0.718	1.60	0.212	0.97	0.329	0.45	0.503
skull penetration location	0.08	0.779	2.12	0.152	1.01	0.319	1.93	0.171	0.00	0.990
Bird type. Bird age	1.78	0.182	0.03	0.864	0.17	0.684	2.33	0.133	0.07	0.798
Bird type. Bird weight	2.70	0.100	0.82	0.700	0.06	0.803	0.11	0.745	0.20	0.654

6.4 Discussion

This study evaluated the kill efficacy of three killing methods (MCD, NMCD, and MZIN) on broilers and layers at two stages of production.. The kill efficacy of on-farm killing methods involves three main areas that need to be addressed: reliability, humaneness and practicality. Due to constraints of bird availability the “slaughter age” broilers were 5 weeks of age at the time of killing, rather than 6 weeks. This resulted in the maximum bird weight of this group being lower than desired (~3 kg), however, the subsequent analysis of the data and previous work (Chapter 5) showed that bird weight did not have a significant effect on kill or device success.

The NMCD and MCD had the highest kill success rates of 100%, compared to the 72% success rate of the MZIN and therefore were deemed the most reliable methods in this study. Erasmus and colleagues (2010a) showed that 100% of turkey hens (N = 26) were successfully killed by mechanical cervical dislocation, re-enforcing the reliability of this method for killing poultry on-farm, but all of those birds displayed a nictitating membrane reflex immediately post device application and maintained this reflex for a mean of 106 s. However, the authors used a Burdizzo (a mechanical cervical dislocation device), which is different to MCD and the NMCD, as it causes dislocation via crushing, not through stretching and twisting (Erasmus et al., 2010a). Crushing injury caused by mechanical cervical dislocation methods is a cause for welfare concern as birds may die of asphyxiation rather than cerebral ischemia, resulting in them showing signs of consciousness for longer (Gregory et al., 1990; HSA, 2004). Another study showed that 100% of broilers (N = 8) were successfully killed by mechanical cervical dislocation using a killing cone, which uses a stretch and twist action similar to that of MCD and NMCD (Gregory et al., 1990). However, in one of the cases the spinal cord was not severed, despite dislocation occurring (Gregory et al., 1990), reducing the likelihood of a concussive effect (Shi and Pryor, 2002; Shi and Whitebone, 2006; Solomon, 1990; UFAW, 2010).

When the NMCD and MCD were applied, they did not require any aiming, unlike the MZIN which meant that a kill success was easier to achieve. MCD does not require any equipment and once trained is relatively simple to apply on birds under 3 kg. For this trial and previous experiments reported in this thesis, MCD was performed by a trained and competent individual (i.e. the author); therefore some of the concerns around the use of this method were not evaluated (e.g. difficulty to learn technique) and this is reflected in the 100% kill success rate. As MCD is purely based on the operator's ability, it is easy to see how issues of fatigue (AVMA, 2007; Canadian Council on Animal Care in science, 2010; Kingsten et al., 2005) could jeopardise its successful application, although results from this study demonstrate that the order in which the birds were killed did not affect kill success, suggesting killing up to 18 birds per day and 90 birds in one week did not affect kill success. Mortality rates (including on-farm emergency killing) in broilers and layers per day can vary substantially and are affected by several factors (e.g. disease, age, strain, etc.); therefore it is difficult to ascertain whether stock-workers would need to kill more than 18 birds per day. Anecdotal evidence suggests that broiler stock-workers could be required to kill more than this number, while layer hen stock-workers are unlikely to do so. Some stock management practices (e.g. flock depletion) will require several hundred or thousand birds to be killed, therefore in such circumstances this study cannot determine if fatigue would affect kill success at such high numbers. The NMCD glove provides the correct position to hold the bird's head in place and generates the stretch and twisting action, which for an inexperienced individual may be beneficial. Like MCD, it had a 100% kill success rate. Therefore the presence of the glove did not hinder the application of the technique, however as both had 100% kill success rate, it cannot be concluded that NMCD was more successful in terms of killing compared to MCD. All birds that underwent MCD or NMCD would immediately wing flap and leg paddle vigorously post-application and an obvious internal gap in the neck, between two cervical vertebrae, could be felt.

The damage caused by the MZIN to the bird's head results in primary and secondary brain injuries; causing brain contusions, hemorrhaging and axonal damage, all of which disrupt brain function and can cause brain death (Claassen et al., 2002; Kushner, 1998; White and Krause, 1993). Successful kills by the MZIN resulted in extensive trauma to the forebrain and the cerebellum. This affected the functioning of several systems e.g. motor systems (unconscious and conscious), cognition, respiration and reflexes (Solomon, 1990; Whittow, 2000). The extent of axonal damage is correlated with the amount of the brain damaged (Krause et al., 1988; White and Krause, 1993), therefore the more extensive the brain damage, the more axons are damaged. Axonal damage has been linked to the length of concussion and unconsciousness (Kushner, 1998; White and Krause, 1993). Skill was required to aim the device and successful judgment in applying reasonable force in order to prevent the device re-coiling, as well as securing the bird's head in place. If this was not achieved there was a reduction in the penetration depth of the bolt, which resulted in insufficient brain damage to cause death. This is highlighted by the result that approximately 42% of birds which were unsuccessfully killed by the device (7/17 unsuccessful kills) did not sustain any skull damage, as the head was either missed completely or only a glancing blow was sustained, which caused only soft tissue damage to the neck or eyes; or recoil resulted in insufficient power to penetrate the skull. When the skull was not damaged, the post-mortem data highlighted the lack of visible brain damage sustained, with the majority of the birds having a score of "none" to every brain region and with the remaining few birds receiving a maximum score of "low"; showing only light bruising and bleeding around a specific brain region, However, it is important to note that the method for assessing brain damage for this study was done by crude observation of the brain regions and attributing a categorical score (e.g. none, low, mid and max). Microscopic damage to the brain tissues and cells (e.g. tearing of nerve fibres and *contrecoup* damage) will not have been visible and several studies have noted the significant affect this type of damage can cause to localised areas of the brain, resulting in impairment of cell function as well as cell death (Comi et al., 2009; Drew and Drew, 2004; Gurdijan and Gurdijan, 1976; Strich, 1970; White and Krause, 1993). Takahashi and colleagues (1981) demonstrated that closed-head trauma and resulting intraparenchymal haemorrhage caused an extensive depolarisation of surrounding brain cells through the accumulation of extracellular K^+ and intracellular Ca^{2+}, affecting the phospholipase activation of the cell and therefore possibly impairing the multiple functions specific to the particular brain region (Krause et al., 1988; Vink et al., 1988). Thus, even though some birds were classified as "unsuccessful" kills, this does not mean that they did not suffer some brain damage, which if the birds had not been emergency euthanised may have been apparent through a prolonged death or abnormal behaviour (Krause et al., 1988; White and Krause, 1993). Such brain damage could also affect the birds' state of consciousness, although it is impossible to say whether the birds were fully unconscious or able to perceive pain after being shot. However, the minimum damage sustained in a successful kill to one or more brain regions was scored as "mid", suggesting that at least one of the five major brain regions sustained structural damage and surrounding bleeding, as well as inferred microscopic damage, all of which would suggest some resulting functional impairment and therefore a possibility that the bird may not be fully conscious post-application of the MZIN and prior to death (Anderson et al., 2006; Krause et al., 1988; White and Krause, 1993; Whittow, 2000). The MZIN required two operators, one to hold the bird, and other to cock and aim the device, as well as a hard surface to rest the bird on, which could be deemed impractical in an on-farm situation. There was also a health and safety concern with the device, as it is a captive bolt and therefore great care is required during its use, and as such safety equipment must be worn (e.g. gloves, safety goggles) (Pizzurro, 2009a; Pizzurro, 2009b). However, the primary issue with the MZIN device was its low kill success rate of 71.7%, which is not reliable enough for a routine on-farm killing method.

Despite the optimal kill success rate for the MCD and the NMCD, the device success rates were significantly lower compared to that of the MZIN. With the MZIN, 43/60 birds were successfully killed and 42 of those birds also achieved device success, therefore when the method was applied correctly, it achieved an optimal effect on the bird. This could be an artefact of the definition of device success for each kill treatment, as there are more specific requirements for NMCD and MCD (e.g. full dislocation at C0-C1, spinal cord severance and both carotid arteries severed, and no skin tears), while for MZIN it was simply penetrating the skull and causing ≥ 1 regions of the brain a minimum score of “mid” range damage.

MCD performed the worst in terms of device success (27%) due to a lower percentage of birds having both carotid arteries severed and the lower percentage of dislocations being at C0-C1 compared to the NMCD, although both dislocation methods had less than 50% device success. Severing of one or more carotid arteries minimises the reduction in blood flow to the brain (Aslan et al., 2006; Perry et al., 2012; Whittow, 2000) and results in a reduction of arterial pressure and eventual cerebral ischemia and/or hypoxia (Gregory and Wotton, 1986; Gregory and Wotton, 1990; Solomon, 1990). However, even if the carotid arteries were not completely severed, the stretching trauma results in narrowing and occlusion of the carotid arteries which may have the same effect as severing them (LeBlang and Nunez, 2000; Solomon, 1990; Whittow, 2000). Both NMCD and MCD caused trauma to both carotid arteries, although did not always sever them. Severing ≥ 1 carotid arteries did not significantly affect the duration of measured reflexes or death-related behaviours, however the number of carotid arteries severed did have a tendency to affect jaw tone, with a significant difference between zero arteries severed compared to one or two. Jaw tone is suggested to be an indicator of consciousness (Croft, 1961; Erasmus et al., 2010a; Erasmus et al., 2010c; Sandercock et al., 2014); therefore birds which had one or two carotid arteries severed were more likely to lose consciousness faster post device application. However, this relationship was only a tendency and severing both carotid arteries did not appear to have an effect on kill success, as this was 100% for both NMCD and MCD.

The duration of the presence of reflexes and death-related behaviours may be over-estimated due to the methodology of measuring their presence every 15 s. In relation to bird welfare, it is better to over-estimate rather than under-estimate, however this limitation could be responsible for post-mortem measures (e.g. number of carotid arteries severed) having no relationship with the durations. For example, if a bird had jaw tone for two seconds post-kill, it was scored as present for the first 15 s interval, and was considered the same as another bird which may have had jaw tone for 14 s. The number of carotid arteries severed may have affected durations of reflexes within the first 15 s; however this could not be measured due to constraints of measuring seven behaviours, which took between 10-15 s. As a result of this study and the findings presented in a previous Chapter (Chapter 5), it appears that the gold standard of severing two carotid arteries may not necessarily be required for minimising time to unconsciousness and brain death, however, it is important to sever at least one in order to reduce blood flow to the brain and spinal cord (Bilello et al., 2003; Dimar et al., 1999; Pryor and Shi, 2006; Shi and Pryor, 2002). Although severing two carotid arteries could still be considered as best practice, it may not be a necessity for defining MCD or NMCD as successful in application.

A further action of cervical dislocation methods is to disrupt blood supply to the top of spinal cord and the brain stem, which causes functional impairment and could result in neurogenic shock (Dumont et al., 2001a; Dumont et al., 2001b). The aim to achieve dislocation of the neck at C0-C1 was to ensure the damage and severing of the spinal cord occurs very near to or at the brain stem, enhancing the likelihood of concussion resulting in disruption to brain stem function and localised temporary or permanent biochemical changes within the neural axons (Brieg, 1970; Gregory et al., 1990; Shi and Pryor, 2002).

More than 80% of birds killed with both MCD and NMCD achieved a C0-C1 dislocation, so the likelihood of trauma to the brain stem was high. Gregory & Wotton (1990) demonstrated that 6/8 birds displayed changes and a reduction in their visual evoked responses post application of cervical dislocation, suggesting a loss of consciousness and all of these birds had dislocations at C0-C1.

Another important reason that device success was not achieved for NMCD and MCD was due to the production of skin tears as a result of the stretching and twisting of the neck. Less than 10% of all birds (MCD – 6.7%; NMCD – 8.3%) received skin tears and even less exhibited external blood loss. The loss of blood into the surrounding environment causes two problematic issues; disease risk and general cleanliness, therefore it is desirable to minimize this. In addition, personal communications with several poultry stock-workers emphasised their distaste for cervical dislocation methods resulting in decapitation or blood loss. NMCD was not more likely to cause skin tears or external blood loss compared to MCD, demonstrating no advantage in this regard for either method.

Durations of reflexes have been used and validated for inferring consciousness in killing assessments of several animals, including poultry (Erasmus et al., 2010a; Erasmus et al., 2010c; McKeegan et al., 2013b; Sandercock et al., 2014). There were no significant differences between killing methods on durations of rhythmic breathing and nictitating membrane and both were lost rapidly post-kill, suggesting both brain death and therefore unconsciousness occurred rapidly for all killing methods. Loss of pupillary reflex is used as a conservative measure for brain death and complete insensibility (Erasmus et al., 2010c; Heard, 2000; Sandercock et al., 2014), and the MZIN had the shortest durations for pupillary reflex compared to NMCD and the MCD, however this only occurred in birds killed successfully with the MZIN (43/60 birds). 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 and Drew, 2004; White and Krause, 1993). Mature layer hens (irrespective of age) exhibited longer durations for pupillary reflex 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 was required to cause immediate loss of reflexes. The bolt is approximately 6mm in diameter, therefore for a small lightweight bird (~500 g), with skull width ranging between 20-26 mm (Chapter 3.2), the bolt directly damaged approximately a quarter of the skull and the brain tissue beneath it. However, in a larger bird (1-2 kg), the skull size ranges from 26-37 mm (Chapter 3.2), therefore it is more difficult to directly damage a wide area of the brain, although secondary damage could impact surrounding brain regions (Kushner, 1998; White and Krause, 1993). The pupillary reflex is affected by disruption to 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 (Blackman et al., 1986; Erasmus et al., 2010c), therefore the pupillary reflex durations for the NMCD and the MCD may be inadvertently elongated (Bilello et al., 2003; Gregory and Wotton, 1990; Perry et al., 2012; Sharma et al., 2005). However, it is important to note that more than 75% of all birds across all killing methods showed pupillary reflex in the first 15 s post-application of a kill treatment, suggesting that none of the devices caused immediate brain death.

The MZIN was associated with significantly shorter jaw tone durations than NMCD or MCD, which has been used as an indicator of consciousness (Croft, 1961; Erasmus et al., 2010a; Erasmus et al., 2010c), suggesting that MZIN caused birds to lose consciousness faster than the other two killing methods. In broilers, NMCD resulted in shorter jaw tone durations compared to MCD and there was a significant effect of bird age, which was confounded with bird type, as all broilers were less than 5 weeks of age, despite being heavier birds than mature layer hens. This could be explained by the fact that late production broilers and mature layer hens were heavier birds and therefore have a greater volume of blood and larger blood vessels, which could make it more difficult to stop or minimise blood flow to the brain stem, which controls jaw tone (Solomon, 1990; Whittow, 2000). MCD and NMCD did cause sufficient damage to the brain stem across all birds, demonstrated by short mean durations for jaw tone, as well as less than 40% of birds ever showing the reflex. In the experiment described in Chapter 5 (See 5.3.5), jaw tone did not correlate with EEG parameters associated with unconsciousness (e.g. F50 < 12.7 Hz or 6.8 Hz) or cerebral inactivity. However, Sandercock and colleagues (2014) showed that unconsciousness induced by anesthetic was associated with loss of jaw tone in layers and turkeys and was a consistent measure of loss of consciousness in this context.

The ceasing of clonic death-related behaviours (e.g. leg paddling and wing flapping) has been used as an indicator of time of death for poultry which are killed by CO_2 gas stunning (Gerritzen et al., 2007), and based on this, all three killing methods were shown to kill birds in similar time periods. The majority of birds showed convulsive wing flapping and leg paddling, which has been observed in several other studies of killing with various methods (Abeyesinghe et al., 2007; Lambooij et al., 1999a; McKeegan et al., 2007). The onset of cloacal movement appears to be the last reflex to be observed before all movements cease. In a small number of birds cloacal movement was not seen, however this was primarily due to the bird defecating and obscuring the visibility of the behaviour.

The NMCD method has shown the most potential as a mechanical killing device for killing poultry on-farm in comparison with MZIN. NMCD matched the performance of MCD, with an equal 100% kill success rate. NMCD had an advantage over MCD in terms of increased likelihood of device success being achieved (e.g. it was more likely to sever carotid arteries), which has been linked to reduced latencies to unconsciousness (refer to Chapter 5.3.3), reduced reflex durations post-application and brain death, which can be viewed as more humane and more practical, with birds dying more quickly, causing less of a delay to confirm successful kills. The NMCD device was also shown to be more consistent than MCD in terms of the physiological trauma it produced, demonstrating that this mechanical method may reduce variability across operators.

In conclusion, these findings suggest that the NMCD device was the most effective killing method compared with the traditional method of MCD. Thus, the NMCD represents a tool which maintains the kill success of MCD, but improves the technique and consistency of its application. After application of NMCD, birds were likely to die from cerebral ischemia due to severing of carotid arteries and were likely to become unconscious rapidly due to extensive trauma to the brain stem and/or spinal cord. The MZIN device had a kill success rate of only 72%, but when successful, was shown to have the fastest loss of cranial reflexes and behaviours (which indicate loss of consciousness and brain death). However, the differences between killing methods were not always significant and were numerically small; therefore it can be concluded that all three killing methods caused rapid brain death and loss of consciousness. Importantly, only NMCD and MCD can be considered to be humane due to their 100% success rate and inducement of rapid reflex loss; indeed a high proportion of birds never showed reflexes at all post-application. Collectively, these results suggest that NMCD is the most promising device in terms of kill success rate (reliability), humaneness and consistency, and it was selected to be taken forward into commercial trials in comparison with MCD across multiple operators.

7 Commercial trials of a Novel Mechanical Cervical Dislocation device for killing poultry on-farm

7.1 Introduction

The success of a despatching device can be defined in three ways; humaneness, reliability and practicality. Humaneness and reliability are important for determining the efficacy of the device; however, assessing practicality and user-reliability is also an essential part of the assessment of a device. Such information is vital to inform decisions as to whether it should be marketed to the public and industry. The final stage of this project was to validate the most successful mechanical device in a commercially relevant environment and test its user reliability and consistency, as well as establishing the amount of training that was required in order for stock-workers to become competent in its use.

The previous laboratory-based experiments determined that the NMCD, was the most humane and reliable mechanical method when the device was used by a single operator (the author). The device out-competed all other devices tested (e.g. MARM, MZIN and MPLI) and was consistent at killing birds (> 96% kill success rate). Analysis of EEG activity during killing of lightly anaesthetised birds demonstrated that the birds were unconsciousness (F50 ranges < 12.7 Hz or 6.8 Hz) within a mean of 3.1 s post device application, but in the cases where the device behaved optimally the duration decreased further to between 1.5 – 2.3 s. The rapid loss of consciousness post device application was also supported by the immediate loss of jaw tone post application in more than 61% of conscious birds killed with NMCD. Based on laboratory evidence that the device was humane and effective, it was important for its practical application in a commercial poultry environment (as well as for backyard poultry keepers) to be investigated. In order to be defined as practical the device must meet several criteria: it must be inexpensive, easily maintained, portable, and simple to use. Any device not meeting most or all of these criteria is unlikely to be adopted by poultry keepers and stock-workers for their standard despatch method (replacing MCD). The NMCD device is a relatively simple glove device with minimal mechanical parts; therefore it can be defined as portable and easily maintained. The cost of equipment and materials to manufacture the single device was approximately £8; therefore it could be marketed at significantly lower cost than the majority of currently available and humane mechanical killing devices. The aim of this experiment was determine ease of use of the device and the amount of training required for poultry stock-workers.

7.2 Materials and Methods

7.2.1 Animal Housing

The experiment was conducted between April and May 2014 on two commercial farms (one broiler and one laying hen) in Scotland. A total of 1120 birds (*Gallus gallus domesticus)* were used; 560 hens (58 weeks old, Hy-Line strain) and 560 mixed-sex slaughter-age broilers (38 days old, Ross 308 strain). This design could result in bird type being confounded with sex, however previous research (Bader et al., 2014; Erasmus et al., 2010a) suggests that sex does not have a significant effect, while bird weight has been shown to have a substantial effect. Therefore the inclusion of male broilers allowed the variation caused by bird weight to be evaluated.

The birds were kept and managed in their on-farm commercial conditions until killing occurred. The layer hens were housed in enriched colony cages (Tecno Cages®, Tecno Poultry Equipment Spa, EU), of 80 birds per colony. The birds had ab libitum access to food and water and environmental controls were automated and in accordance with Laying Hen Codes of Recommendations (DEFRA, 2002a). The broilers were housed in large deep litter (wood-shavings) floor pens with *ab libitum* access to food and water. The stocking density in each pen varied due to the flock being depopulated for slaughter at the time of the trial, however remained in accordance with the Broiler (meat chicken) Codes of Recommendations (DEFRA, 2002b).

7.7.2 Study Design

The trial was designed around a 2 x 2 factorial design, with a total of eight stock-workers (four per farm (i.e. bird type) being assessed on their performance with the NMCD device and MCD. The NMCD was assessed for its kill efficacy, user reliability and training requirement alongside a control method (MCD) in a commercial environment and multiple operators. The device design is described in detail in section 3.3.4. The NMCD device had been tested in previous experiments on cadavers (refer to Chapter 4), unconscious (refer to Chapter 5) and conscious birds (refer to Chapter 6) and had demonstrated its ability to kill birds consistently and humanely across broiler and layer type chickens, albeit when applied by a single operator. The MCD technique used was dependent on stock-worker previous training and onsite standard operating procedures and did not always follow HSA's guidelines (HSA, 2004). In general, MCD was performed in one swift movement; the operator pulled down on the bird's head, stretching the neck, while rotating the bird's head upwards into the back of the neck.

The trial was conducted in two batches (one batch per farm location/bird, i.e. layer hen farm/broiler farm). Each batch involved the sampling of 70 birds per killing method (NMCD/MCD) per stock-worker (N = 4) across two days, giving a total of 560 birds per batch. Each stock-worker performed both killing methods (NMCD/MCD) within a day, with kill order and killing method systematically randomised (Table 7.1). Due to restrictions on stock-worker availability on-farm, only one stock-worker performed a killing method at a time, with another assisting by collecting birds. The killing of birds for these trials was not classed a regulated procedure under the Animals (Scientific Procedures) Act 1984 so the number of birds selected per killing method, and their body weight (< 3 kg), was the maximum allowed for MCD by the current European Council Regulations on the Protection of Animals at the Time of Killing (European Council, 2009). The work did however adhere to the 3Rs principal as all birds were destined for slaughter or to be culled irrespective of being included in the trial. All birds were weighed and identified with a numbered leg tag (numbered 1 to 70 for kill order) prior to killing.

Table 7.1 Timetable of killing method orders for each stock-worker across the two experimental days per farm.

Bird type/Stock-worker		DAY 1				DAY 2			
		AM		PM		AM		PM	
Broilers	1	MCD	@	NMCD	@				
	2	@	NMCD	@	MCD				
	3					NMCD	@	MCD	@
	4					@	MCD	@	NMCD
Layer hens	5	NMCD	@	MCD	@				
	6	@	MCD	@	NMCD				
	7					MCD	@	NMCD	@
	8					@	NMCD	@	MCD

Note: @ = stock-worker assisting (e.g. collecting birds etc)

The eight male stock-workers selected for the trial were experienced in performing MCD on a regular basis and were deemed competent by their on-site farm manager. Biometric measures of all stock-workers were recorded (e.g. hand span, hand length, arm length, height and weight), as well as their handedness and MCD technique. A flow chart of the experimental procedure for each killing method is shown in Figure 7.1. In order to assess the training requirements for NMCD and the kill efficacy of each killing method for each stock-worker, the 70 birds per killing method were sub-divided into three tests: Test 1 – applied to 10 cadavers; Test 2 – applied to 30 live conscious birds; and Test 3 – applied to 30 live conscious birds (Table 7.2). There was no set maximum time for completion of the tests, but the time for completion of each was recorded. The killing rate of birds within each test was not controlled in an attempt to reduce any stress on the stock-worker through time pressure and allow them to perform the killing method at a comfortable rate. Between tests 5 minute breaks were provided in an attempt to standardise rest periods between the tests.

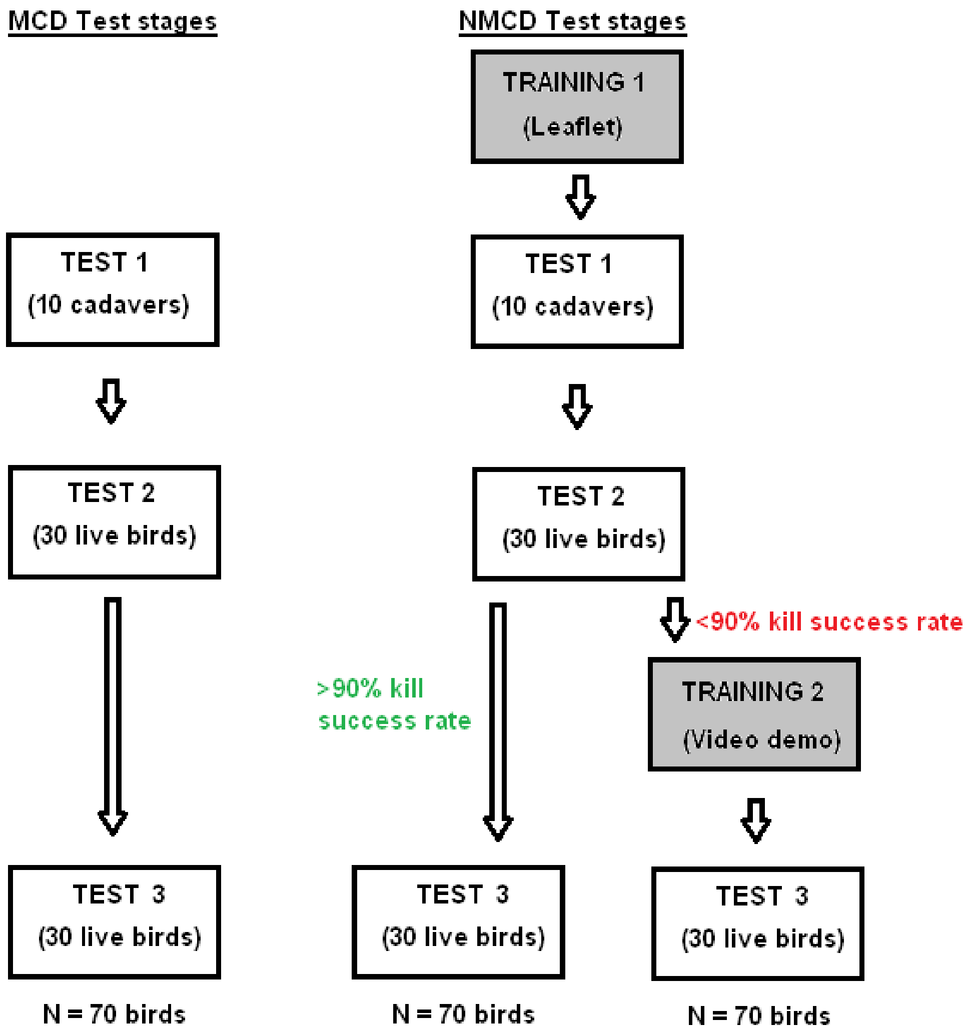


Figure 7.1 Flow chart of the experimental procedure for both killing methods.

Table 7.2 Number of birds allocated across the two killing methods and the three tests per stock-worker.

		Broilers				Layer hens			
Stock-worker		**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**
Test 1	*NMCD*	10	10	10	10	10	10	10	10
(Cadavers)	*MCD*	10	10	10	10	10	10	10	10
Test 2	*NMCD*	30	30	30	30	30	30	30	30
(Live birds)	*MCD*	30	30	30	30	30	30	30	30
Test 3	*NMCD*	30	30	30	30	30	30	30	30
(Live birds)	*MCD*	30	30	30	30	30	30	30	30
TOTAL N		140	140	140	140	140	140	140	140

Kill efficacy was determined by a trained experienced poultry technician immediately post application of a method through confirmation of death by cervical dislocation (e.g. cessation of rhythmic breathing, loss of nictitating membrane, jaw tone, gap in the neck present) and observation of only one kill attempt (i.e. one stretch and twist action). Multiple attempts were recorded as a fail, even if they resulted in the death of the bird. In Test 1, kill efficacy on the cadaver birds was established by the confirmation a gap between two cervical vertebra via externally feeling the neck and no greater than one attempt to achieve this. In Tests 2 and 3, if the birds did not display signs of immediate loss of consciousness and death post-application (e.g. cessation of rhythmic breathing, loss of nictitating membrane, jaw tone and onset of clonic leg and wing convulsions), they were immediately emergency euthanised via MCD by the poultry technician (in practice, this was never required). If at any point during the tests the stock-workers became uncomfortable to continue, the test was halted and for NMCD only additional training was offered, depending on the training stage completed (Table 7.3). The tests continued following additional training if the stock-worker was happy to continue; otherwise the tests were permanently halted.

Table 7.3 Description of the three training stages and under what circumstances they are provided.

Training stage	Description	Training provided
1	Leaflet	Prior to Test 1
2	Video demo (2 minute video with voice over)	- If Test 2 overall kill efficacy was between 50% and 90%, training provided prior to Test 3. - If Test 2 kill efficacy reaches 15/30 birds unsuccessfully killed, training provided within test as emergency intervention. - If stock-worker requests halt to either Test 2 or 3 due to unease in continuing, irrespective of kill efficacy, training provided if training 1 has been completed.
3	One-to-one training by trained technician (maximum time of 5 minutes).	- If Test 3 kill efficacy reaches 15/30 birds unsuccessfully killed, training provided within test as emergency intervention - If stock-worker requests halt to test due to unease in continuing, irrespective of kill efficacy, training provided if training 1 and 2 has been completed.

No training relating to standard MCD killing was provided prior to testing and the three tests were performed consecutively, with 5 minute breaks following Test 1 and Test 2. In Test 1, 10 birds were euthanised prior to testing in the predetermined test order via a sodium pentobarbital injection (Euthatal, Merial Animal Health Ltd., Essex, UK), at a dosage of 1 ml per 1 kg of bird weight. In Tests 2 and 3, the killing method was applied to 30 live and conscious birds. Kill efficacy was recorded for each bird and overall efficacy was calculated for each test.

All stock-workers underwent the first training stage for NMCD, immediately prior to Test 1, which involved being given the NMCD device and leaflet on the device and how to use it (Appendix 3). Each stock-worker was given a maximum of 5 minutes to read the leaflet and try on the various sizes of the device (small/medium/large) in order to select the appropriate size for his hand. Following the training leaflet the stock-worker was given 10 cadavers to perform the NMCD method on. This allowed the stock-worker to become accustomed (e.g. adjusting hand grip) to the device without compromising bird welfare. Kill efficacy was recorded for each bird and at the end of Test 1 the stock-worker was asked if he was comfortable to continue to the next test. If he answered yes, then following the 5 minute break the stock-worker continued on to Test 2. If the stock-worker answered no, the experiment was halted and no further birds were killed. Previous results (Chapter 3) demonstrated that application of killing methods on cadavers may affect their application due to the lack of muscle tone, therefore no additional training prior to Test 2 was provided. For Test 2 the stock-worker was given 30 live and conscious birds to kill with the relevant method. If the overall kill efficacy was between 50% and 90%, training stage 2 (video demo – refer to Table 7.3) was provided before progressing to Test 3. However, if kill efficacy reached less than 50% in Test 2 (15/30 birds), training stage 2 was instigated at this point before the remaining birds were killed. At the end of Test 2 the stock-worker was asked if he was comfortable to continue to Test 3. If he answered yes, then following a 5 minute break the stock-worker continued. If the stock-worker answered no, he was offered additional emergency training (one-to-one training – refer to Table 7.3), and if following this he remained uncomfortable the experiment was halted and no further birds were killed. In Test 3 the stock-worker was given 30 live and conscious birds to kill with the relevant method. Following each application the bird was confirmed dead (e.g. cessation of rhythmic breathing, loss of nictitating membrane, jaw tone and onset of clonic leg and wing convulsions) by a trained technician and the kill efficacy recorded. If the kill efficacy reached less than 50% (15/30 birds), the test was halted and the final training was offered (one-to-one training) prior to the remaining birds being tested.

7.2.2.1 Post-mortem measures

A post-mortem examination was performed on every bird immediately after the application of the killing method in Test 1 and after confirmation of death in Tests 2 and 3. For all killing methods, binary yes/no measures were recorded for skin broken, external blood loss, subcutaneous hematoma, dislocation of the neck, vertebra damage (e.g. intra-vertebra dislocation/break), and whether the spinal cord was severed. The level of cervical dislocation was recorded (e.g. between C0-C1, C1-C2, C2-C3, etc.), as well as a measurement of the length (cm) of gap between the dislocated cervical vertebra. The number of carotid arteries severed was also recorded as zero, one or both. Any birds which underwent emergency euthanasia as a result of a failed kill could not undergo post-mortem as the data on the anatomical damage produced was confounded by the emergency MCD.

7.2.2.2 Questionnaire

At the end of the tests, irrespective of whether they were completed, each stock-worker was asked three yes/no questions. The first question asked whether they found the NMCD device helpful in dislocating birds’ necks; the second asked whether they preferred the NMCD device over the MCD method; and the third asked whether they would consider using the NMCD device as an on-farm killing method if it were made available, as a replacement for the now restricted MCD method.

7.7.3 Statistical Analysis

Data collected at the bird level and stock-worker level and were summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat (14^{th} Edition). Statistical significance was termed by a threshold of 5% probability based on F tests. Summary graphs and statistics were produced at the stock-worker level. For all models the random effects included the date and stock-worker. All fixed effects were treated as factors and classed as categorical classifications. Results were statistically significant at $P \leq 0.05$, and tendencies at $P \leq 0.10$.

Generalised Linear Mixed Models (GLMMs) using logit link function and binomially distributed errors due to the nature of the binary data were used to statistically compare kill efficacy across stock-workers. In the maximal models, fixed effects included killing method, bird type, training level, bird order, session, handedness, technique and all their interactions. Dispersion was fixed at one. Summary statistics and graphs were summarised at the stock-worker level.

As kill performance was dependent on number of kill attempts as well as generating a gap between two cervical vertebra, birds which were scored as “no” for kill, were not excluded from GLMM analysis of post-mortem measures, but instead kill performance was incorporated as a factor into the model. Logit link function and binomially distributed errors were used due to the nature of the binary and categorical data, in order to compare post-mortem measures and their consistency across stock-workers. For the size of neck gap variable, distribution was normal and the logit link function not used. Dispersion was fixed dependent on the variable (e.g. dislocation level – dispersion fixed at seven; skin broken – dispersion fixed at one). Maximal models included several fixed effects: killing method, kill, bird type, training level, bird order, session, weight, number of kill attempts, and all their interactions. Some variables were also included as factors in modelling for other variables (e.g. variable = dislocation level, factor = neck gap size).

7.2.3.1 Post-mortem data

Analysis of post-mortem binary measures (e.g. skin break, subcutaneous hematoma, etc.) and categorised measures (e.g. cervical dislocation level, number of carotid arteries severed, etc.) was conducted via GLMMs using logit link function and binomial distribution. Fixed effects included were killing method, kill, bird type, training level, bird order, session, bird weight, number of kill attempts, and all their interactions. Bird age was not included as it was confounded with bird type. In some cases, variables were also included as factors in modelling for other variables (e.g. variable = dislocation level, factor = neck gap size).

7.2.3.2 Questionnaire

The three questions were designed to only provide a basic insight into the stock-workers personal evaluation of the NMCD device. All stock-workers were asked identical questions in the same order. Frequency differences in the binomial (yes/no) data for the answers to all three questions across the eight stock-workers were analysed using Chi-Square tests in Minitab 15, with the expected observations assumed as all “no” as the NMCD device had not be used or seen by the stock-workers prior to the trial. Further statistical analysis of stock-workers sub-divided by bird type were not undertaken due to low sample size (i.e. N = 4).

7.3 Results

Variation between stock-worker biometric measures was minimal (Table 7.4) with handedness evenly split across the eight stock-workers, although there was a bias towards left handedness (3/4 stock-workers) on the broiler farm and right handedness on the layer farm (3/4 stock-workers). All sizes of NMCD were chosen and used by the stock-workers, despite the minimal hand size variation, with the majority of stock-workers choosing the “Large” sized glove (5/8 stock-workers). Five out of eight stock-workers used the “Two-finger” method for dislocating chickens’ necks, which was defined as when the bird’s head was held in the palm of the operator’s hand, with the neck in-between the index and middle fingers. The remaining stock-workers used the “Ring” method which was defined as when the bird’s head was held in the operator’s palm, with the neck in-between the index finger and thumb of the operator, creating a ring shape around the bird’s neck, however the two methods were bird type specific, with the only broiler stock-workers using the “Ring” method.

Table 7.4 Mean and standard errors of the stock-worker biometric measures (N = 8).

Biometric measure	Mean	Standard Error (±)
Hand span (cm)	19.8	0.3
Hand length (cm)	20.6	0.4
Arm length (cm)	67.6	4.5
Weight (kg)	92.1	8.0
Height (m)	1.8	0.2

7.3.1 Kill Performance

Individual stock-worker performance through the three tests are summarised in Table 7.5. The overall mean stock-worker kill performance was significantly higher for the MCD (98.4 ± 0.5%) killing method compared to NMCD (81.6 ± 1.8%) ($F_{1,8}$ = 38.28, P < 0.001). Performances were classed as unsuccessful if greater than one kill attempt was required; MCD had a lower mean and maximum number of kill attempts (mean = 1.01 ± 0.01, maximum = 2.00) compared to NMCD (mean = 1.26 ± 0.03, maximum = 5.00).

Bird type also had an effect on kill performance, irrespective of killing method, with a better mean kill performance in layer hens (88.4 ± 7.5%) compared to broilers (81.5 ± 12.3%) ($F_{1,8}$ = 4.22, P = 0.041). However there was also an effect of the interaction between killing method and bird type; with laying hen kill performance being higher compared to broilers with NMCD (broilers = 63.1 ± 21.9%; layer hens = 80.0 ± 14.2%). The opposite interaction was apparent with MCD (broilers = 100.0 ± 0.0%; layer hens = 96.8 ± 1.6%) ($F_{1,8}$ = 4.45, P = 0.035). Training level required for NMCD had a significant effect on kill performance ($F_{2,8}$ = 6.76, P = 0.038) (Figure 7.2), but there were no other significant interactions between other factors and training level (e.g. time of day, bird type, etc.). However, there was a significant interaction between training level required and technique ("Ring" or "Two-fingers"), with stock-workers who used the "Ring" technique requiring more training (training level mean = 2.3 ± 0.7) than stock-workers who used the "Two-fingers" technique (training level mean = 1.2 ± 0.2). Handedness had no effect on kill performance and neither did the interaction between killing method and handedness.

Table 7.5 Stock-worker performance, training required and agreement to each test for both killing methods, sub-divided by bird type.

Killing method	Bird type	Stock-worker	Training level	Test agreement (Y/N) 1	2	3	Test kill performance record 1	2	3
MCD	Broiler	1		Y	Y	Y	10/10	30/30	30/30
		2		Y	Y	Y	10/10	30/30	30/30
		3		Y	Y	Y	10/10	30/30	30/30
		4		Y	Y	Y	10/10	30/30	30/30
	Layer	5		Y	Y	Y	10/10	30/30	29/30
		6		Y	Y	Y	10/10	28/30	27/30
		7		Y	Y	Y	10/10	30/30	30/30
		8		Y	Y	Y	9/10	30/30	28/30
NMCD	Broiler	1	1	Y	Y	Y	7/10	27/30	30/30
		2	3	Y	N	N	9/10	1/5	-
		3	1	Y	Y	Y	10/10	29/30	27/30
		4	3	Y	N	N	0/10	-	-
	Layer	5	1	Y	Y	Y	10/10	30/30	30/30
		6	2	Y	Y	Y	0/10	9/30	18/30
		7	1	Y	Y	Y	10/10	30/30	28/30
		8	1	Y	Y	Y	2/10	30/30	27/30

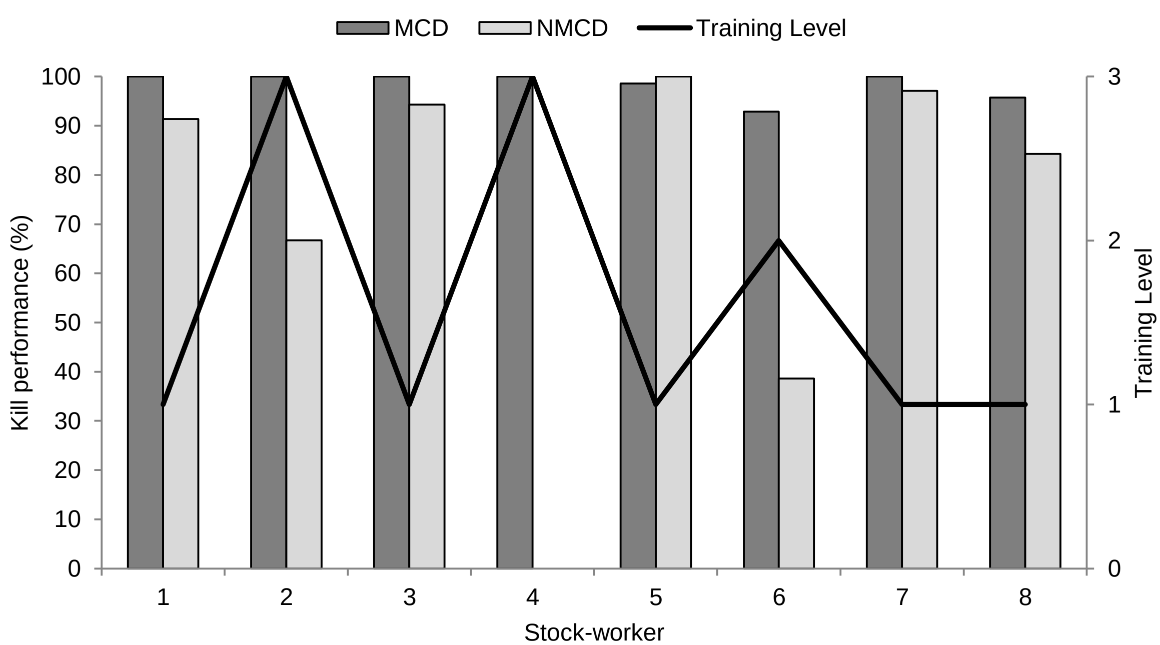


Figure 7.2 Stock-worker total kill performance (%) for each killing method in relation to the NMCD training level required during NMCD testing. Stock-workers 1-4 worked with broilers and stock-workers 5-8 worked with layer hens. Refer to Table 7.5 for varying number of birds killed per stock-worker and killing method.

Bird order had an effect on kill performance ($F_{1,8}$ = 8.73, P = 0.003), with lower kill success being associated with birds killed early in the test compared to birds killed later (Figure 7.3a). However, the interaction between killing method and bird order also demonstrated that MCD kill performance decreased as more birds were killed ($F_{1,8}$ = 6.83, P = 0.009) (Figure 7.3b), while the opposite effect was seen for NMCD, with performance improving (Figure 7.3c). Similar to bird order, session (AM/PM) had an effect ($F_{1,8}$ = 5.65, P = 0.018) and so did the interaction between session and killing method ($F_{1,8}$ = 5.26, P = 0.022), although day did not ($F_{1,8}$ = 0.03, P = 0.889). Overall kill performance increased in the afternoon session (PM: 0.95 ± 0.01) compared to the morning (AM: mean = 0.87 ± 0.02), however the interaction demonstrated that there was no difference between session for the MCD (AM: 0.99 ± 0.01; PM: 0.98 ± 0.01) while for NMCD, kill performance was better in the afternoon session compared to the morning (AM: 0.72 ± 0.03; PM: 0.91 ± 0.02).

a)

b)

c)

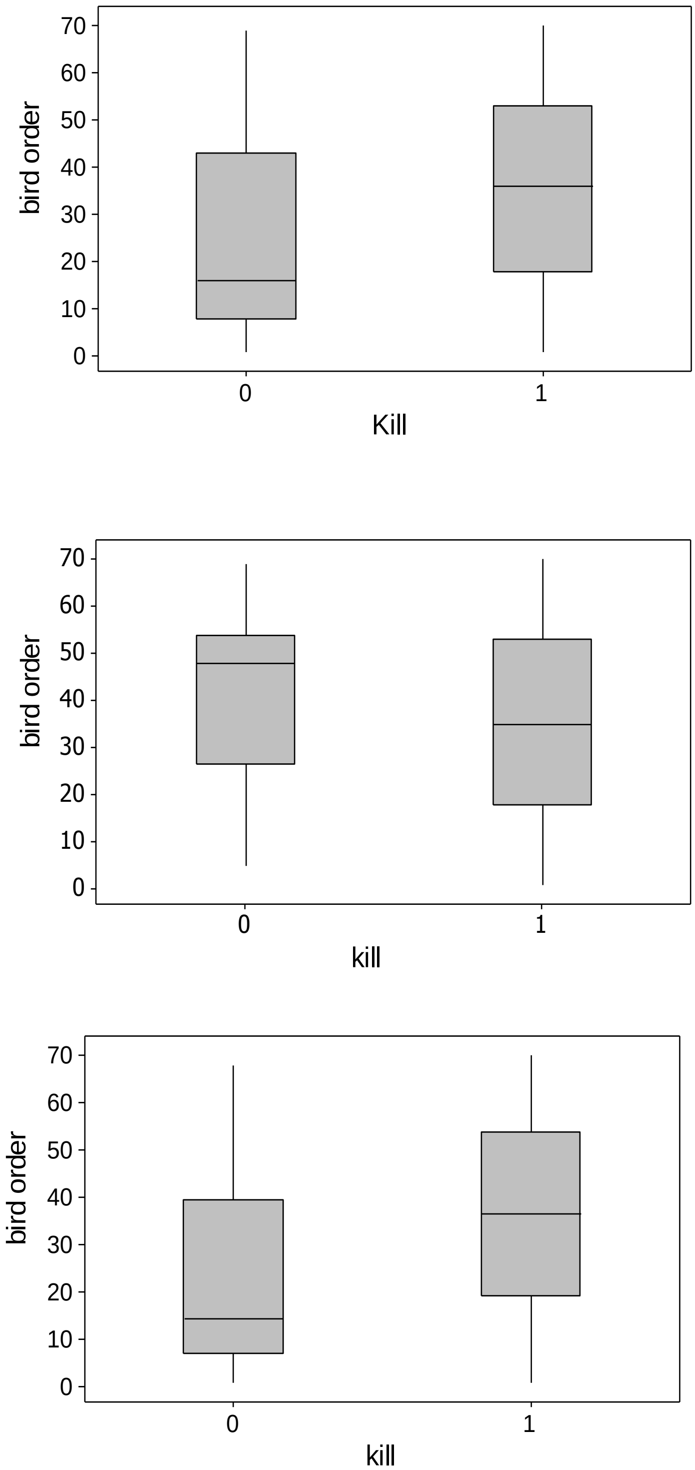


Figure 7.3 Range of kill performance dependent on bird order for (a) both killing methods (overall (N = 1006)); (b) MCD only (N = 560); and (c) NMCD only (N = 446). Successful kills were categorised as "1", and unsuccessful kills as "0".

7.3.2 Post-mortem measures

The calculated means (±SE) for the majority of post-mortem measures, at the stock-worker level, are shown in Figure 7.4 and Figure 7.5. For the remaining binary measures (yes/no); there was no variation across all stock-workers, irrespective of killing method, with all achieving 100% cervical dislocation, subcutaneous hematoma and spinal cord severed in all birds. There was also no variation in vertebral damage with all stock-workers causing none in any birds irrespective of killing method.

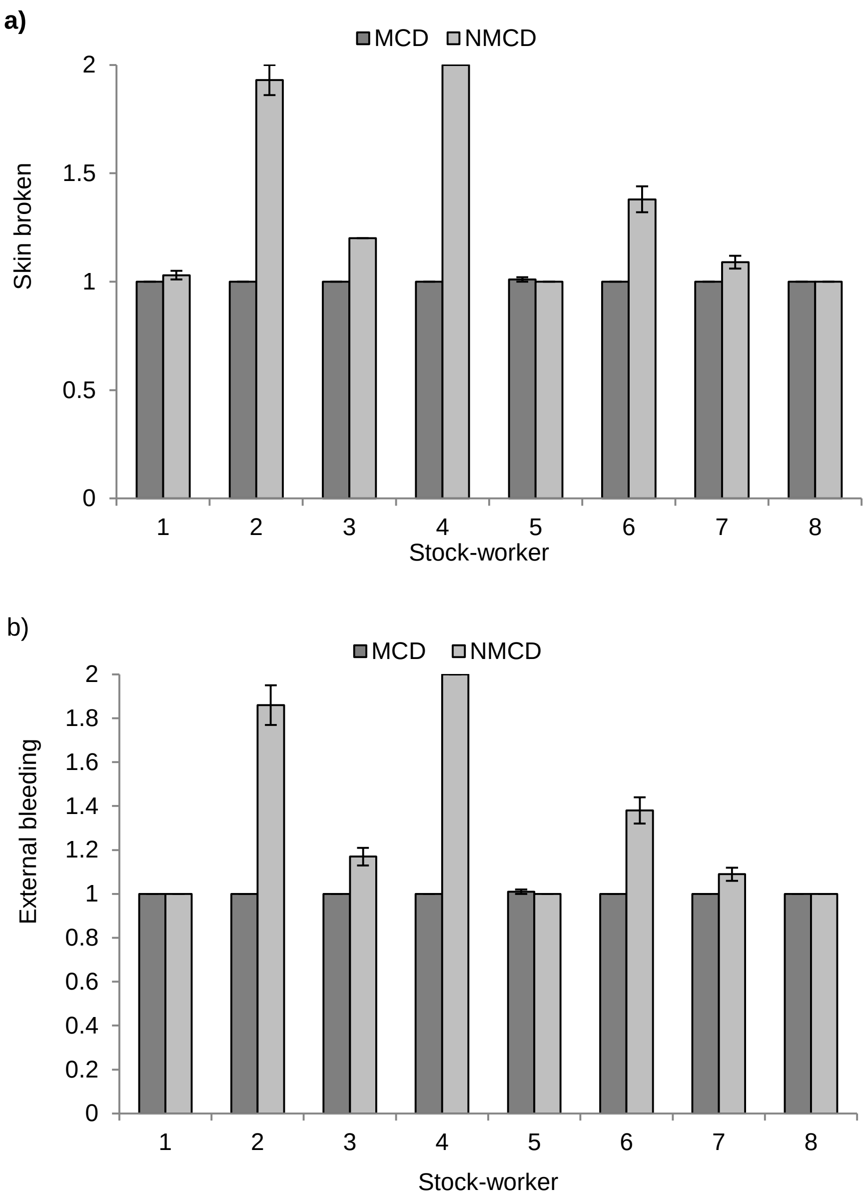
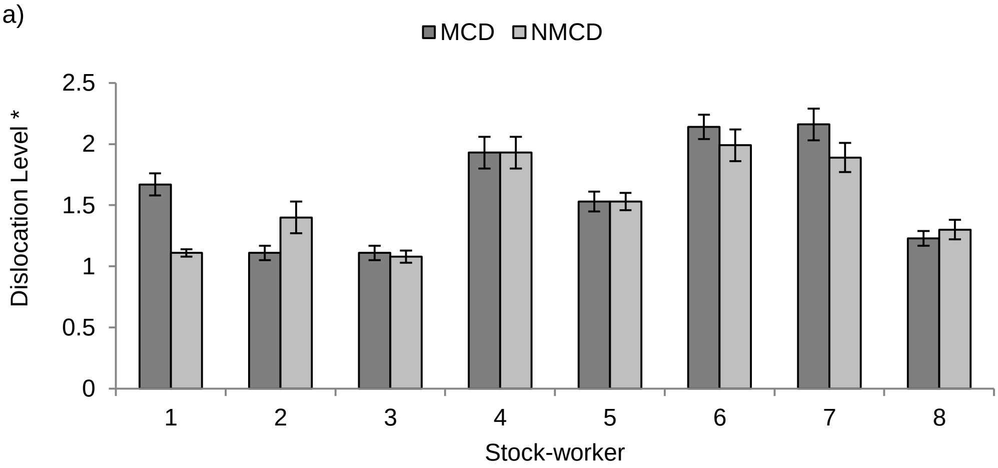
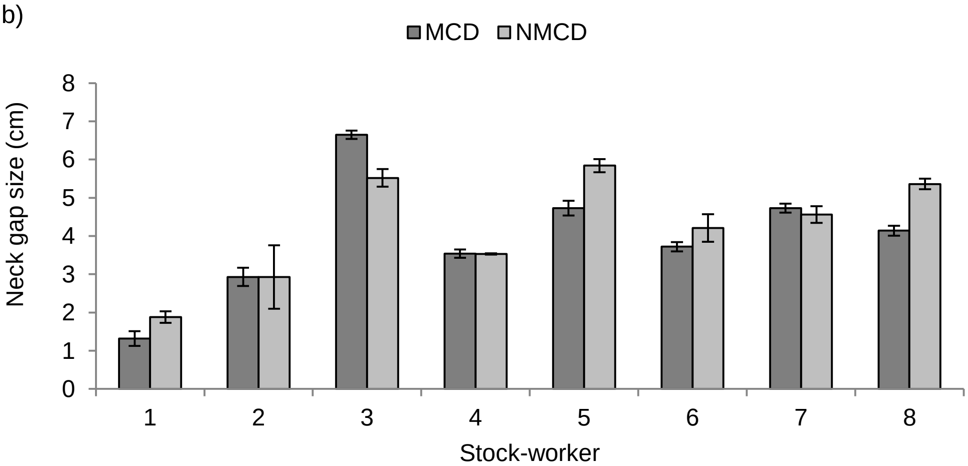


Figure 7.4 Comparison of means and standard errors (±) for binary (yes/no) measures for (a) skin broken and (b) external bleeding across all stock-workers and killing methods. Binary means were calculated by converting yes/no levels to numerical categories (e.g. no = 1; yes = 2).

a)



b)



c)

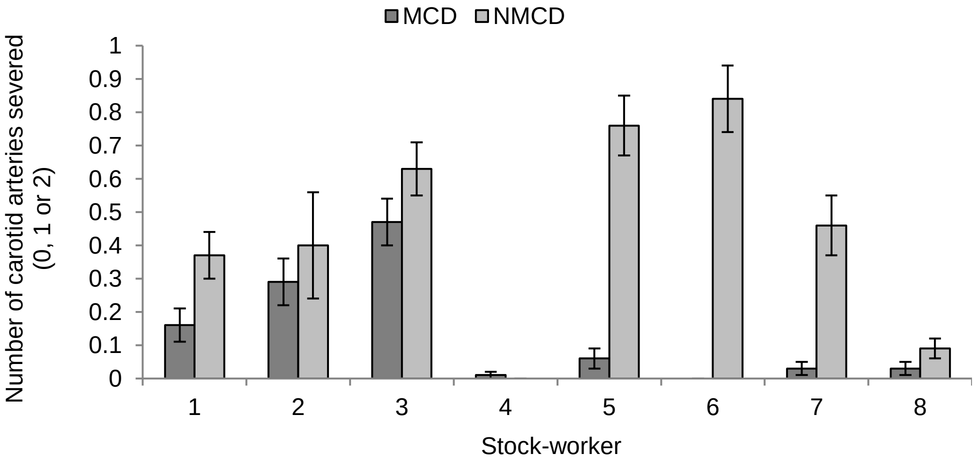


Figure 7.5 Comparison of means and standard errors (±) for (a) dislocation level; (b) neck gap size (cm); and (c) number of carotid arteries severed (0, 1 or 2) for both killing methods across all stock-workers. Dislocation level(*) means were calculated by converting vertebral levels to a numerical category (e.g. C0-C1 = 1; C1-C2 = 2; C2-C3 = 3; C3-C4 = 4; C4-C5 = 5; C5-C6 = 6; and C6-C7 = 7).

GLMM analyses could not be run on the post-mortem measures with no variation (e.g. dislocation occurred and vertebral damage which were 100% and 0% respectively). For all other post-mortem measures, killing method had an effect and this is summarised in Table 7.6. The NMCD method was more likely to sever a carotid artery, achieve a higher dislocation level (e.g. C0-C1), and cause a larger neck gap size compared to MCD.

Table 7.6 Descriptive statistics (mean, SE, minimum, and maximum) as well the GLMM results for comparison of all post-mortem measures by killing method (MCD or NMCD).

Post-mortem measure	Killing method	Mean	SE	Min.	Max.	df	*F statistic*	*P value*
Number of carotid arteries severed	MCD	0.13	0.02	0.00	2.00	1,8	9.97	<0.001
	NMCD	0.51	0.03	0.00	2.00			
Dislocation occurred+ $	MCD	2.00	0.00	2.00	2.00			
	NMCD	2.00	0.00	2.00	2.00			
Dislocation level*	MCD	1.62	0.04	1.00	6.00	7,8	10.6	0.002
	NMCD	1.44	0.04	1.00	7.00			
External bleeding+	MCD	1.04	0.00	1.00	2.00	1,8	96.32	<0.001
	NMCD	1.15	0.02	1.00	2.00			
Neck gap size (cm)	MCD	3.96	0.08	0.00	8.00	1,8	16.25	<0.001
	NMCD	4.41	0.11	0.00	10.00			
Skin broken+	MCD	1.04	0.00	0.00	2.00	1,8	94.68	<0.001
	NMCD	1.16	0.02	0.00	2.00			
Spinal cord severed+ $	MCD	2.00	0.00	2.00	2.00			
	NMCD	2.00	0.00	2.00	2.00			
Subcutaneous hematoma+ $	MCD	2.00	0.00	2.00	2.00			
	NMCD	2.00	0.00	2.00	2.00			
Vertebral damage+ $	MCD	1.00	0.00	1.00	1.00			
	NMCD	1.00	0.00	1.00	1.00			

* *Dislocation point means were calculated by converting vertebral levels to a numerical category (e.g. C0-C1 = 1; C1-C2 = 2; C2-C3 = 3; C3-C4 = 4; C4-C5 = 5; C5-C6 = 6; and C6-C7 = 7).*

\+ *Binary yes/no means were calculated by converting yes/no levels to numerical categories (e.g. no = 1; yes = 2).*

$ *No variation therefore GLMMs not analysed.*

Dislocation level was not affected by whether the kill was successful (defined in Section 7.2.2) ($F_{1,8}$ = 0.41, P = 0.524), bird order ($F_{2,8}$ = 1.47, P = 0.480), or the interaction between killing method and bird order ($F_{2,8}$ = 0.44, P = 0.642). Bird type had an effect on the dislocation level with higher levels (e.g. C0-C1) more likely to occur in broilers (mean = 1.36 ± 0.04) than in layers (mean = 1.67 ± 0.04). The interaction between killing method and bird type also had an effect (demonstrated in Figure 7.6). For NMCD, training level also had an effect with the highest dislocation levels achieved at training level 2 (mean = 1.16 ± 0.04) compared to level 1 (mean = 1.56 ± 0.03) and 3 (mean = 1.56 ± 0.08). The bird number also had an effect ($F_{1,8}$ = 4.51, P = 0.034), but the interaction with killing method did not ($F_{1,8}$ = 3.48, P = 0.062). Lower dislocation levels were more likely at the start of the tests and within the first test (cadavers) (mean = 1.75 ± 0.03) compared to the later tests (Test 2 = 1.56 ± 0.04; and Test 3 = 1.49 ±0.04). Lower dislocation levels were significantly more likely to occur in morning sessions (AM = 1.44 ± 0.04) compared to afternoon sessions (PM = 1.63 ± 0.04) ($F_{1,8}$ = 40.64, P < 0.001). However, session also had a significant interaction with killing method ($F_{1,8}$ = 94.90, P < 0.001) (Figure 7.7), with stock-workers who performed in the afternoon session for NMCD achieving higher dislocation levels than stock-workers who performed in the morning sessions, however the opposite was seen in MCD stock-workers.

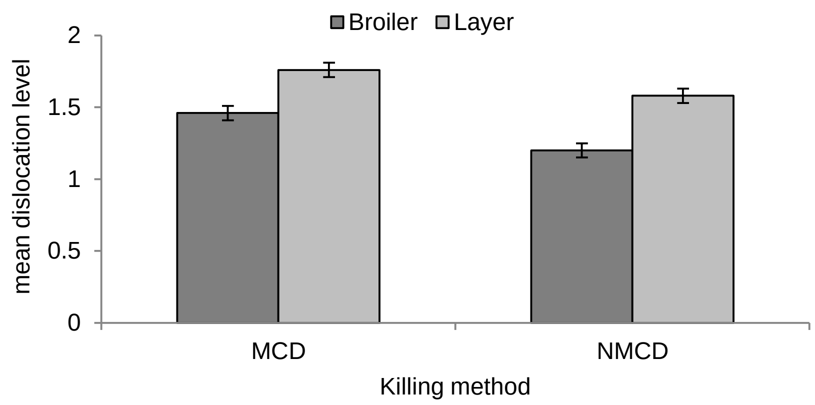


Figure 7.6 Mean and SE (±) dislocation levels showing the interaction between killing method and bird type.

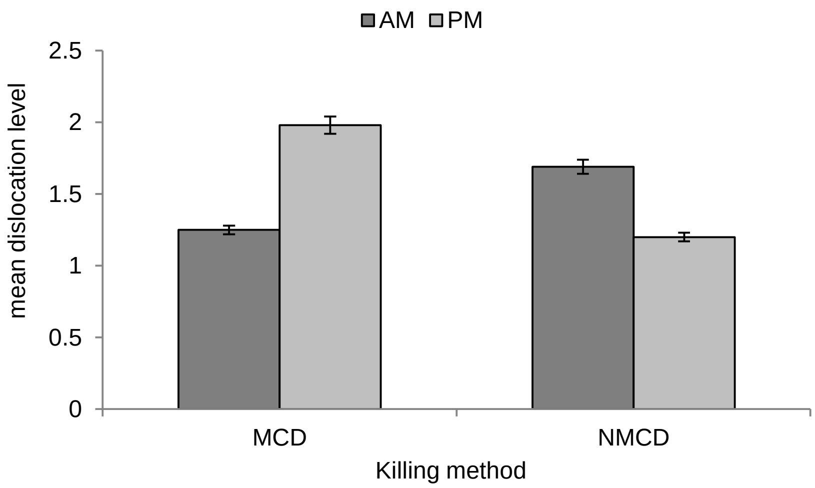


Figure 7.7 Mean and SE dislocation levels showing the interaction between killing method and session (AM/PM).

Bird weight also had an effect on dislocation level ($F_{1,8}$ = 4.13, P = 0.042), with lower dislocation levels occurring in lighter birds (Figure 7.8). Neck gap size ($F_{1,8}$ = 73.7, P < 0.001) and the interaction between it and killing method ($F_{1,8}$ = 4.30, P = 0.038) had an effect on dislocation level, with larger neck gap sizes occurring with higher dislocation levels, with NMCD out-competing MCD, by producing larger neck gap sizes for high dislocation levels compared to MCD (Figure 7.9).

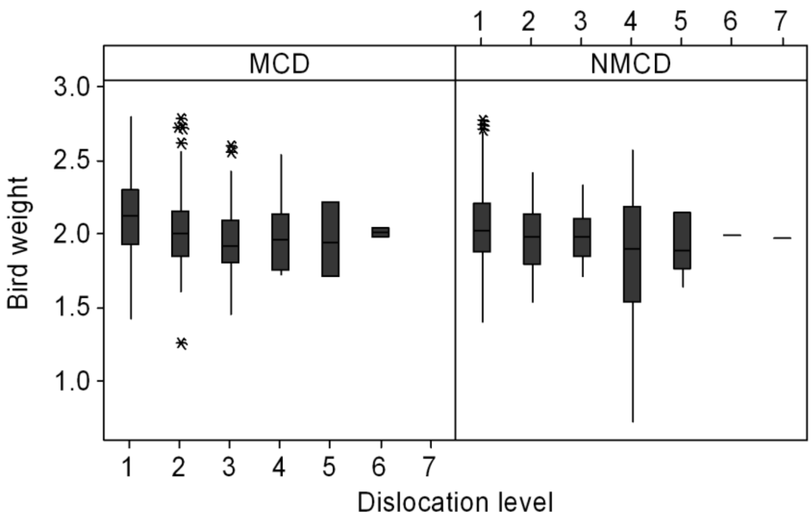


Figure 7.8 Effect of bird weight on dislocation level for both killing methods.

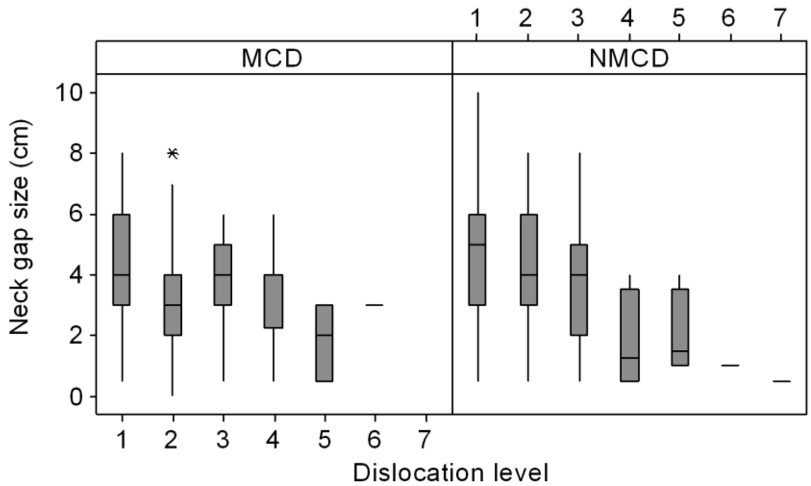


Figure 7.9 Effect of neck gap size (cm) on dislocation level for both killing methods.

Neck gap size was not affected by kill success ($F_{1,8}$ = 1.16, P = 0.281), or number of kill attempts ($F_{4,8}$ = 2.00, P = 0.092). Bird type had an effect ($F_{1,8}$ = 4.28, P = 0.039), and an interaction with killing method ($F_{1,8}$ = 4.50, P = 0.034) on neck gap size, with layer hens exhibiting larger neck gap sizes (mean = 4.67 ± 0.07 cm) than broilers (mean = 3.52 ± 0.12 cm). The same relationship was apparent in each killing method, but for NMCD there was a larger difference between neck gap sizes dependent on bird type compared to the MCD (Figure 7.10). For NMCD, training level also had an effect on neck gap size ($F_{2,8}$ = 12.26, P < 0.001), with the larger neck gap sizes seen at training level 2 (mean = 5.84 ± 0.17 cm) compared to level 1 (mean = 4.26 ± 0.12 cm) and 3 (mean = 2.52 ± 0.50 cm). Bird number ($F_{1,8}$ = 9.89, P = 0.002) also had an effect on neck gap size and was positively correlated (r = 0.10, P = 0.002). Similarly test number (refer to Figure 7.1) also had an effect ($F_{2,8}$ = 15.61, P < 0.001), as did the interaction with killing method ($F_{1,8}$ = 8.05, P < 0.001), demonstrating that neck gap size increased with test number overall, however the interaction with killing method demonstrated that MCD showed no increase of neck gap size with test number, but there was variation between tests (Figure 7.11). However, the NMCD method showed a sharp increase in neck gap size with test number. Session had an effect on neck gap size ($F_{1,8}$ = 14.32, P < 0.001), as did its interaction with killing method ($F_{1,8}$ = 40.67, P < 0.001) (Figure 7.12), which showed that overall, neck gap size was slightly larger in the afternoon session regardless of stock-worker. However when incorporating killing method, MCD showed a decrease in neck gaps size during the afternoon session compared to the morning, with the opposite relationship for NMCD. Bird weight had an effect on neck gap size with heavier birds more likely to exhibit extremes in neck gap sizes (e.g. very small gaps and large gaps) (Figure 7.13).

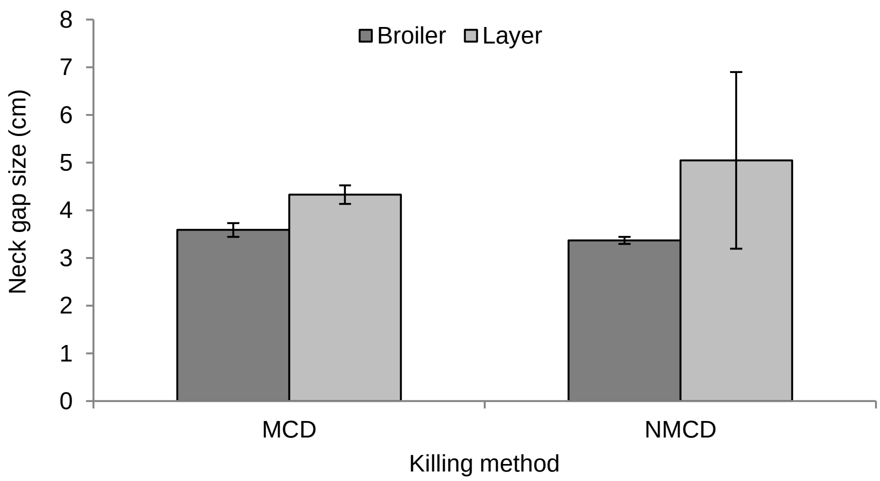


Figure 7.10 Effect of bird type (broiler/layer) on neck gap size (cm) for both killing methods.

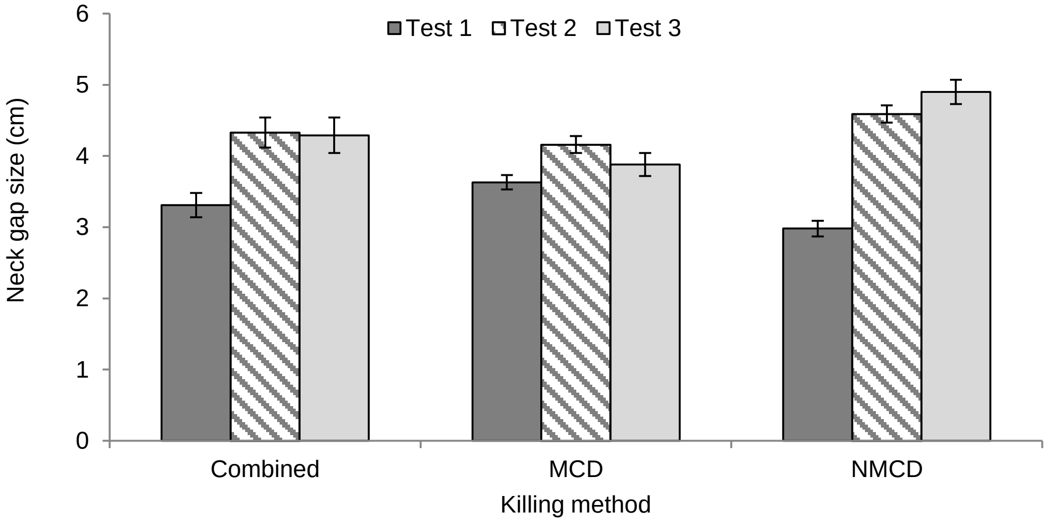


Figure 7.11 Effect of test number on neck gap size for the killing methods combined, as well as the individual killing methods.

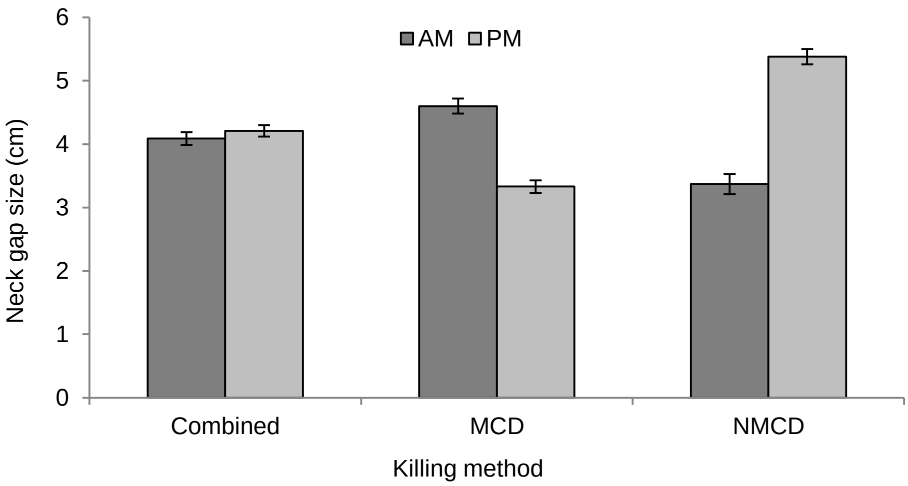


Figure 7.12 Effect of session on neck gap size for combined and individual killing methods.

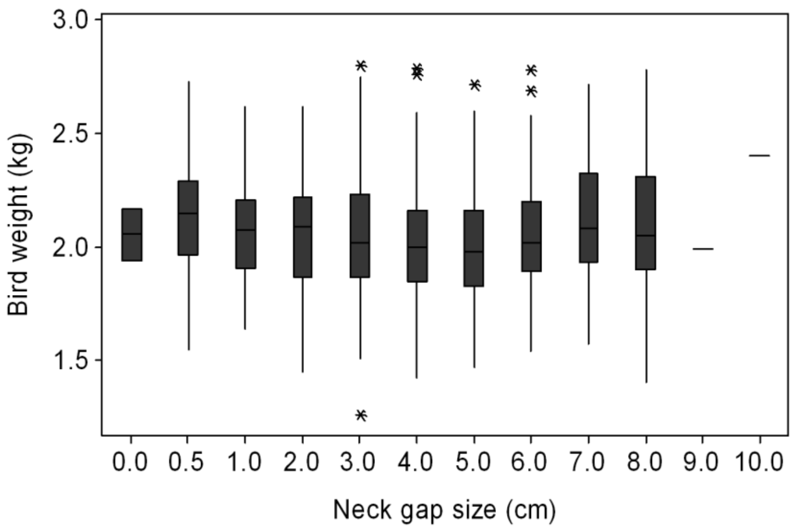


Figure 7.13 Variation in bird weight (kg) in relation to neck gap size (cm).

The number of carotid arteries severed was significantly affected by killing method (Table 7.6), with the NMCD method more likely to sever a minimum of one artery compared to the MCD method. Whether the kill was successful or not had no effect on whether a carotid artery was severed ($F_{1,8}$ = 0.40, P = 0.530), and neither did bird type ($F_{1,8}$ = 0.01, P = 0.979). However, the interaction between killing method and bird type did have an effect ($F_{1,8}$ = 13.23, P < 0.001), with the number of carotid arteries severed higher in layers for NMCD compared to broilers, and the opposite seen with MCD (Figure 7.14a). Bird order had no effect ($F_{1,8}$ = 0.36, P = 0.551) on the number of carotid arteries severed, but the interaction with killing method did ($F_{1,8}$ = 10.77, P < 0.001). There was no effect of bird order for NMCD, however for MCD, the number of carotid arteries severed was higher for birds killed nearer the start of the test. This result was also supported by a significant interaction between killing method and session ($F_{1,8}$ = 4.65, P = 0.032), but not for session ($F_{1,8}$ = 1.11, P = 0.293) or test number ($F_{2,8}$ = 1.40, P = 0.496) as individual factors or the interaction with test number ($F_{2,8}$ = 0.91, P = 0.636). Stock-workers who performed in the morning for MCD performed better than those in the afternoon, but there was no effect for NMCD (Figure 7.14b). The number of severed carotid arteries was higher at the start of tests for MCD, but the opposite effect was seen for NMCD (Figure 7.14c). Bird weight ($F_{1,8}$ = 0.80, P = 0.372), test number ($F_{2,8}$ = 1.40, P = 0.496), dislocation level ($F_{1,8}$ = 1.34, P = 0.248), or number of kill attempts ($F_{4,8}$ = 0.89, P = 0.470) had no effect on the number of carotid arteries severed as individual factors or interactions with killing method. For NMCD tests, training level had an effect ($F_{2,8}$ = 4.28, P = 0.014), with training level 2 showing the highest mean number of carotid arteries severed (mean = 0.76 ± 0.09) compared to level 1 (mean = 0.48 ± 0.04) or 3 (mean = 0.23 ± 0.10). Neck gap size as a factor had an effect on the number of carotid arteries severed ($F_{1,8}$ = 74.45, P < 0.001), with a positive correlation (r = 0.483, P < 0.001), but there was no interaction with killing method ($F_{1,8}$ = 0.04, P = 0.85).

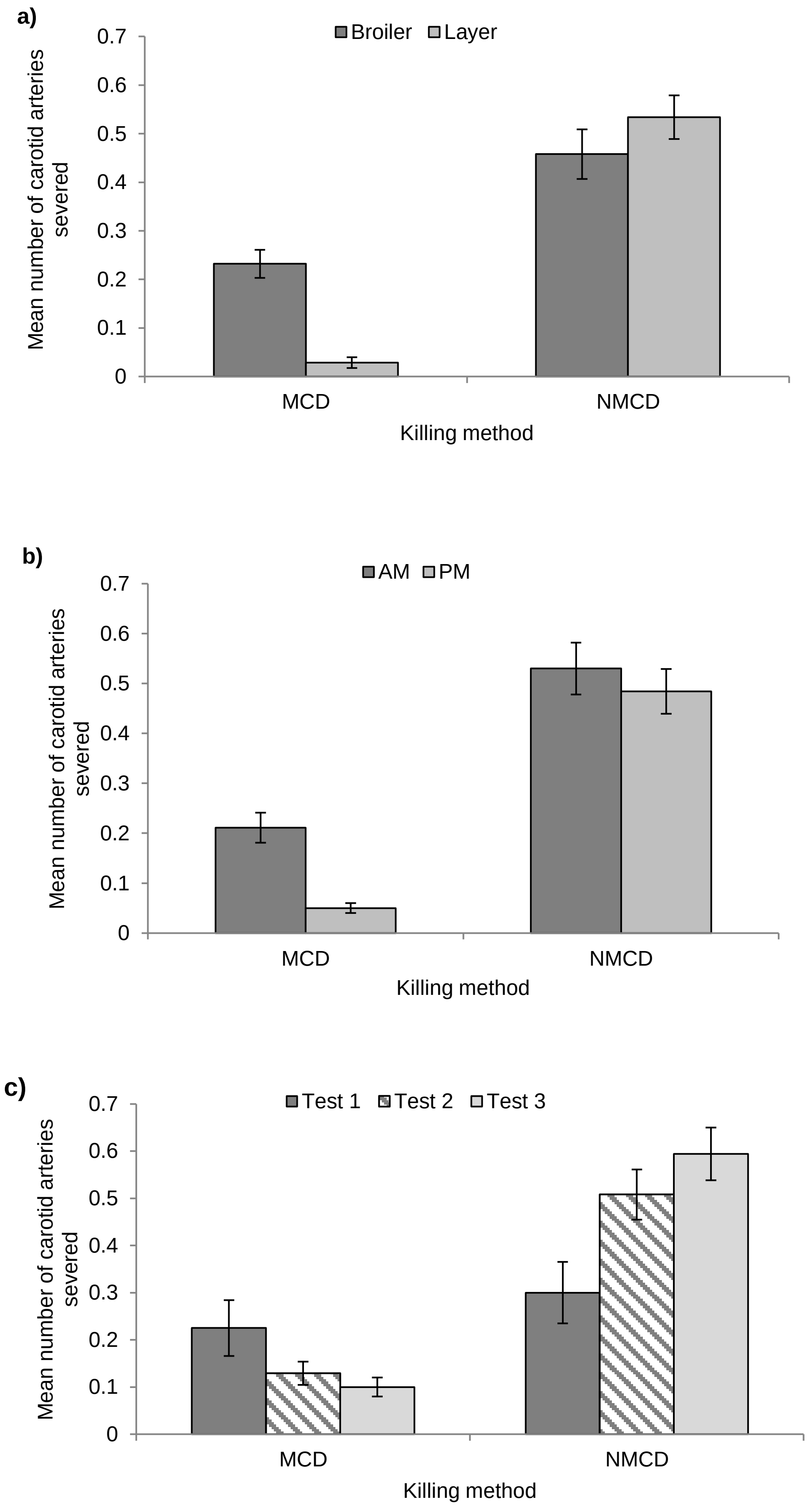


Figure 7.14 Effect of the interaction of killing method with (a) bird type; (b) session; and (c) test number on the number of carotid arteries severed.

Whether or not the skin was broken was affected by kill success ($F_{1,8}$ = 94.78, P < 0.001), with the skin more likely to be broken in unsuccessful kills (kill success: yes = 1.03 ± 0.01; no = 1.49 ± 0.05). Bird type ($F_{1,8}$ = 20.25, P < 0.001) and the interaction between bird type and killing method ($F_{1,8}$ = 18.12, P < 0.001) had an effect, with NMCD in general being more likely to tear the skin compared to MCD and within the NMCD treatment broilers were more likely to have their skin broken during the method application than layers (Figure 7.15). After NMCD, the skin was significantly more likely to be torn when the stock-worker underwent training level 3 (mean = 1.96 ± 0.04 (N = 26)) and 1 (mean = 1.14 ± 0.02 (N = 349)), compared to training level 2 (mean = 1.00 ± 0.00 (N = 70)).

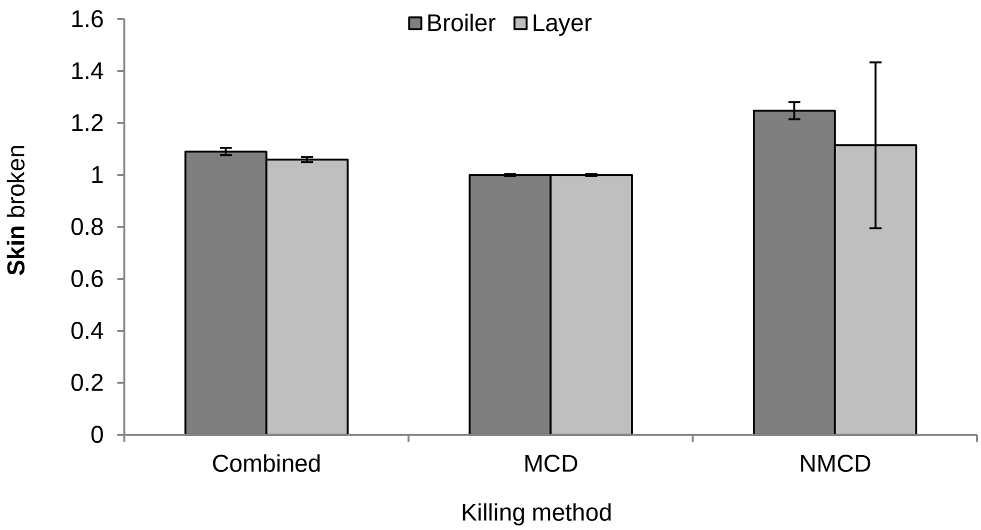


Figure 7.15 Effect of killing method and bird type on whether or not the skin was broken. Binary yes/no means were calculated by converting yes/no levels to numerical categories (i.e. no = 1; yes = 2).

Bird order ($F_{1,8}$ = 9.70, P = 0.002) had an effect on whether the skin was torn, with birds killed earlier in tests more likely to receive skin tears (Test 1 = 1.23 ± 0.03;Test 2 = 1.04 ± 0.01; Test 3 = 1.05 ± 0.01). There was no variation as a result of the interaction between killing method and test bird number ($F_{1,8}$ = 2.82, P = 0.094). Session had no effect on whether the skin was torn ($F_{1,8}$ = 1.83, P = 0.176), but there was an interaction between session and killing method ($F_{1,8}$ = 5.73, P = 0.017), with no variation between sessions for MCD, but with significant variation for NMCD with stock-workers which performed in the morning session much more likely to tear the skin compared to the afternoon (Figure 7.16). The number of kill attempts also had an effect ($F_{4,8}$ = 15.27, P < 0.001), with the greater number of kill attempts being associated with skin tears being more likely

(Table 7.7). There was no effect of an interaction between number of kill attempts and killing method ($F_{1,8}$ = 0.44, P = 0.512). Bird weight had no effect on whether or not the skin was torn during application of either method ($F_{1,8}$ = 0.43, P = 0.513).

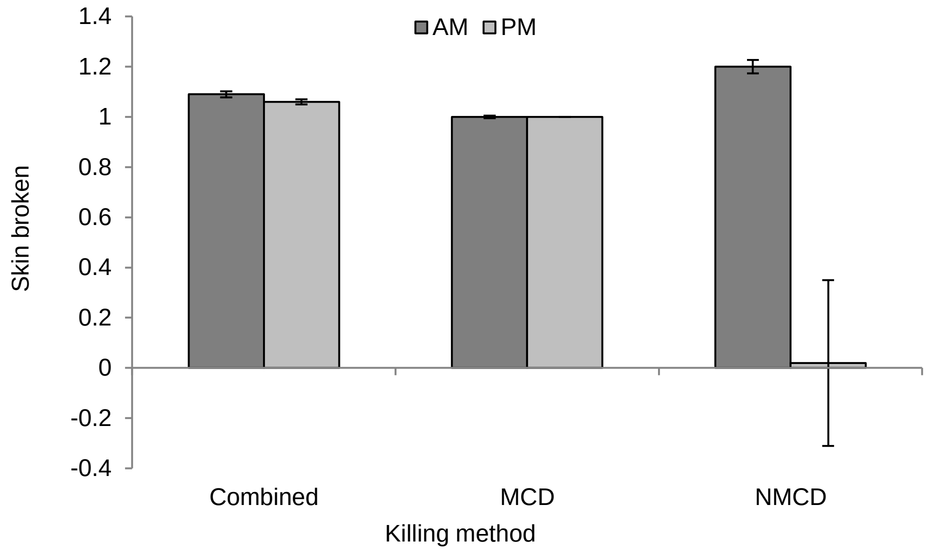


Figure 7.16 Effect of killing method and session on whether or not the skin was broken. Binary yes/no means were calculated by converting yes/no levels to numerical categories (i.e. no = 1; yes = 2).

Table 7.7 Mean, SE, minimum and maximum of whether or not the skin was broken dependent on the number of kill attempts.

Number of kill attempts	N	Mean	SE (±)	Min.	Max.
1	922	1.04	0.01	1.00	2.00
2	57	1.32	0.06	1.00	2.00
3	18	1.78	0.10	1.00	2.00
4	5	2.00	0.00	2.00	2.00
5	3	2.00	0.00	2.00	2.00

Note: Binary yes/no means were calculated by converting yes/no levels to numerical categories (i.e. no = 1; yes = 2).

7.3.3 Questionnaire

The percentage of stock-workers which answered yes or no to each question (see Section 7.2.2.2) is shown in Figure 7.17. Chi-Square tests showed that there were significant differences between the expected and observed counts for each question across the eight stock-workers (P < 0.001).

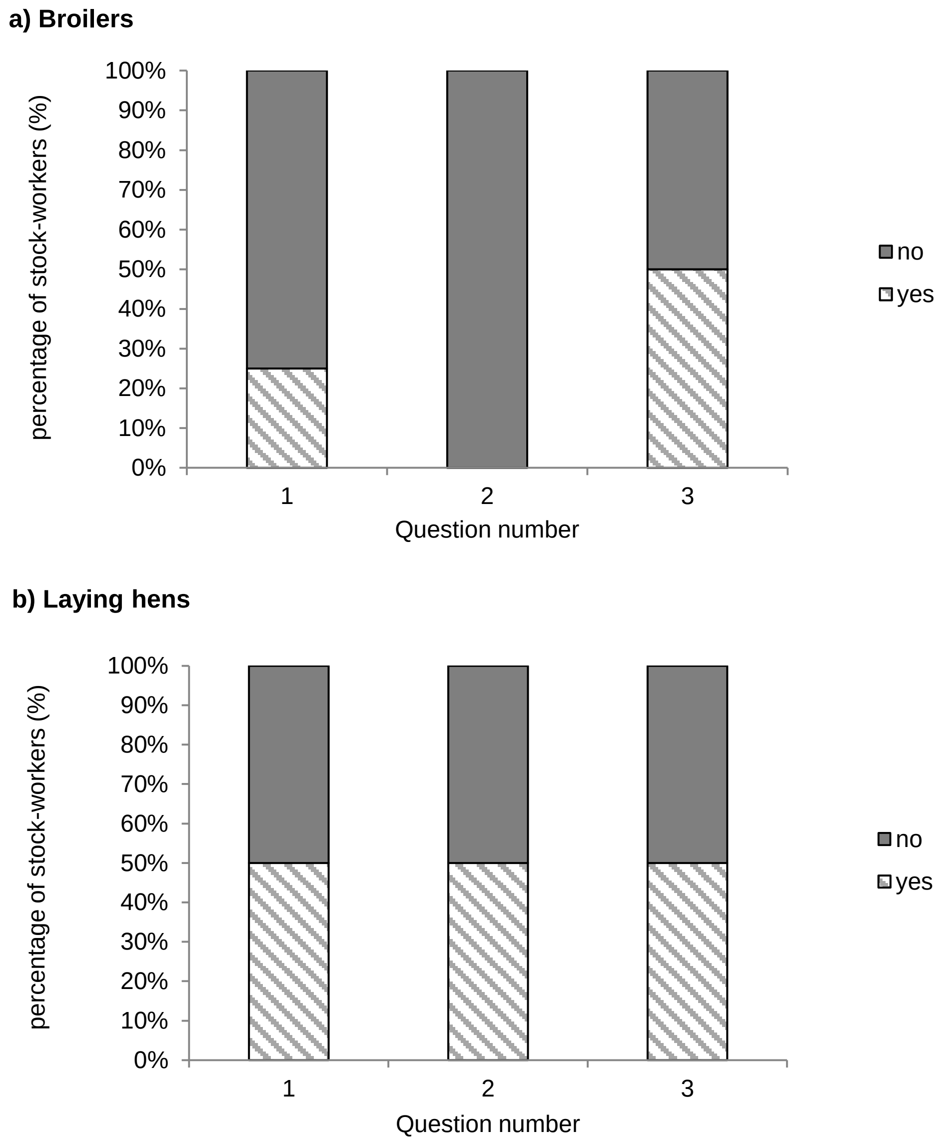


Figure 7.17 Graphical percentages of the binomial (yes/no) data for the three questions asked to stock-workers which worked with (a) broilers; and (b) laying hens following NMCD tests. Chi-Square test results for each question: (1) $\chi^2 = 107.65_{(1,8)}$, $P < 0.001$; (2) $\chi^2 = 46.54_{(1,8)}$, $P < 0.001$; and (3) $\chi^2 = 194.02_{(1,8)}$, $P < 0.001$.

7.4 Discussion

Evaluation of the user-reliability and consistency of a novel killing device in its intended environment is a vital part of its detailed assessment. The NMCD device has previously been evaluated in laboratory environments with one user, where it was demonstrated to produce a high kill success rate and to increase the trauma to the neck (e.g. severing of carotid arteries) compared to MCD and a novel captive bolt device (Modified Zinger) (Martin et al., 2014; Martin et al., 2015). This study aimed to make a practically relevant comparison between the NMCD and standard MCD on a layer hen farm and a broiler farm, with four stock-workers per farm. Evaluation of the methods across the eight stock-workers demonstrated that the NMCD device was not as reliable as MCD for killing poultry in a commercial setting and across multiple users, based on the criteria that a successful kill was defined as no more than one kill attempt, a gap in the neck, and immediate behavioural sign of loss of consciousness and brain death (e.g. cessation of rhythmic breathing).

There was substantial between-stock-worker variation in kill performance, and despite NMCD being designed around the MCD method, the results demonstrated that a high kill performance with MCD did not guarantee a high kill performance with NMCD. However, the NMCD treatment did increase the consistency and scale of physiological trauma to birds' necks, which has been linked to unconsciousness and brain death (Brieg, 1970; Dumont et al., 2001a; Dumont et al., 2001b; Shi and Pryor, 2002; Weir et al., 2002), suggesting NMCD's potential to cause reduced latencies to unconsciousness and brain death compared to MCD (although a previous experiment (Chapter 5) demonstrated no significant difference between latencies of F50 < 12.7 or 6.8 Hz (unconsciousness ranges) for MCD and NMCD). However, previous results showed that when the killing methods were performed optimally (e.g. severed two carotid arteries, dislocation at C0-C1, etc.) the latencies were reduced compared to when the devices performed sub-optimally but were still classed as successful kills (see Chapter 5.3.3).

During the NMCD tests, there was an apparent advantage for stock-workers who performed in the afternoon compared to the morning, and this is most likely due to be due to familiarity because the afternoon stock-workers assisted and observed in the morning, which the morning stock-workers did not experience prior to testing. Post-mortem measures also demonstrated that stock-workers who performed the NMCD treatment in the afternoon showed better performances than stock-workers who performed in the morning, with higher mean dislocation levels, larger neck gap sizes and higher likelihood of one or two carotid arteries being severed. This suggests that there was an advantage to stock-workers who observed the device in use prior to using it themselves. There was no such advantage shown for stock-workers when using MCD, instead a slight disadvantage was shown, with afternoon performances showing a marginal decrease in kill efficacy, which could attributed to fatigue (physical and mental), as they had assisted in the morning (e.g. carrying birds, etc.) prior to their tests in the afternoon.

All stock-workers were experienced in MCD and had been approved as competent by their specific farm managers, providing the MCD treatment with an advantage of prior experience compared to NMCD. An unexpected hurdle for some of the stock-workers was adapting to the NMCD treatment, when their MCD method was not the standard (HSA, 2004) “Two-finger” method, but the “Ring” method instead. The NMCD device is designed around the “Two finger” method providing users who perform MCD in this way with an advantage. This was supported by the increased NMCD training required for stock-workers who used the “Ring” method. Interestingly, the method was bird type specific, with only broiler stock-workers using the “Ring” method. However, this could be as a result of the training they received as part of their on-farm standard operating procedures. Stock-workers which used the “Ring” method had 100% kill success in MCD, but the post-mortem results demonstrated it produced the less severe trauma to the neck compared to the “Two finger” MCD method as well as the NMCD treatment. It was also observed that on rare occasions (27 birds) some of the stock-workers adopted a ‘double pull’ technique when using either treatment, although the majority were for the NMCD treatment, which automatically resulted in bird kill failures. The double pull appeared to be almost a mechanism to “double-check” the dislocation had occurred, with the pulls occurring in rapid succession. This treatment resulted in the death of the bird, however was deemed an application failure. There is no way of knowing whether the first pull resulted in a complete dislocation or not, resulting in potential spinal cord and brain stem damage, which should cause unconsciousness, therefore in theory the second rapid pull (or stretch) may not be a welfare concern. However, it is a concern that the stock-workers felt they had to perform the double pull, perhaps because they were not confident with their application and birds may not have not been fully cervically dislocated on the first attempt, suggesting they may have experienced pain (Bader et al., 2014; Erasmus et al., 2010a; Gregory and Wotton, 1990; Parent et al., 1992; Rutherford, 2002).

For the NMCD treatment the majority of stock-workers only required training level one suggesting the device was fairly intuitive to use following the reading of the leaflet. Only three stock-workers required further training, one was a layer hen stock-worker (level 2) and the other two worked with broilers. Both the broiler stock-workers used the “Ring” method for MCD and reached the maximum training level (one-to-one training) and following this; one chose not to continue due to continued unease with using the device, while the other’s test was cancelled on welfare grounds as there was concern the individual was not concentrating or compiling with the training correctly.

Based on a small sample size, the training levels provided here were not sufficient to train stock-workers who were inexperienced in the “Two finger” method and therefore may not be sufficient to train amateur people in the NMCD method. However, this remains speculation as using the NMCD device to train amateur individuals was not the aim of this study, and it could be that the training levels were not sufficient to re-train individuals to a different method. Despite this, for the majority of stock-workers and irrespective of training level, kill performance and physiological trauma to the birds’ necks (e.g. neck gap size, number of carotid arteries, dislocation level) increased through the NMCD tests for each stock-worker, suggesting performance improvement with practise. The opposite effect was seen with MCD, where stock-workers appeared to slightly decrease in kill their performance over time and there was a substantial lack of consistency in trauma as a result of the MCD treatment application. This was despite the stock-workers being trained in the treatment and deemed competent; highlighting the concern that MCD is not a consistent method, irrespective of training or experience. This inconsistency could be due to slight variations in method application, stock-worker fatigue (including hand/arm muscle fatigue), or lack of concentration over time (i.e. boredom) (DEFRA, 2014; Sparrey et al., 2014).

Interestingly, there was a general trend that kill performance was lower in test 1 (cadavers), irrespective of killing method, which could be attributed to the apparent difficulty in cervically dislocating birds’ necks when there was no resistance due to a lack of muscle tone. This is likely to make it difficult to ascertain when the dislocation has occurred and this also explains the slightly higher number of kill attempts (i.e. multiple pulls/stretches) and over-stretching (i.e. accidental decapitations) in test 1 compared to other tests across both killing methods. These results suggesting that using cadavers as a training aid for MCD or NMCD may be of limited value.

Unlike in previous experiments, broilers had a lower kill success rate compared to laying hens; however this could also be an artefact of the “Ring” method that 3/4 of broiler stock-workers used, as well as two of these stock-workers not completing the live bird tests, therefore reducing their overall kill performance. Previous work has suggested that broilers are easier to cervically dislocate as they are less physiologically mature (McLeod et al., 1964) and therefore their connective tissue is less elastic and has less tensile strength compared to older/mature birds (Vogel, 1980; Vogel, 1986). This is supported by the current results for dislocation level, number of carotid arteries severed, and neck gap size, with layer hen stock-workers performing better than broiler stock-workers. Again, this could be an artefact of the unforeseen confounding factors of bird type and MCD method technique.

There was considerable variation across stock-workers in terms of their consistency of physiological trauma produced as a result of each killing method. The NMCD method did not reduce the likelihood of intra-vertebral damage, or improve the likelihood of a dislocation occurring, or the spinal cord being severed (both 100% of birds). Therefore NMCD did not outperform MCD for these measures. More than 58% of all birds, irrespective of killing method (MCD = 58.6%; NMCD = 69.1%) received a C0-C1 dislocation level, which focusses the physiological damage to the top of the spinal cord and the brain stem. Damage to this area is associated with spinal cord concussion, neurogenic shock and loss of consciousness, suggesting NMCD was more likely to result in birds’ losing consciousness post application than MCD (Dumont et al., 2001a; Dumont et al., 2001b; Freeman and Wright, 1953; Harrop et al., 2001; McLeod et al., 1964; Shaw, 2002; Weir et al., 2002; Whittow, 2000).

Bird weight negatively correlated with dislocation level, which is the opposite effect to that seen in previous experiments within this thesis (Martin et al., 2014; Martin et al., 2015) and other research (Bader et al., 2014; DEFRA, 2014; Erasmus et al., 2010a; Gregory and Wotton, 1990). In these studies, heavier birds were more difficult to cervically dislocate at C0-C1 compared to lighter birds. Larger neck gap sizes were associated with higher dislocation levels. Once the dislocation had been achieved the “follow-through” stretch, which causes the neck gap, demonstrates the ease in dislocating and stretching the birds’ necks. Therefore smaller neck gap sizes could be attributed to difficulty in causing the dislocation which may have limited the “follow-through” stretch. The C0-C1 connection is heavily protected and reinforced by connective tissue and is the join between the skull (occipital condyle) and the top of the spine, with C1 being the smallest cervical vertebra (Bader et al., 2014; Erasmus et al., 2010a; Holdsworth, 1962; McLeod et al., 1964; Whittow, 2000). This makes dislocation between C0-C1 very difficult compared to inter-vertebral dislocation between similar sized and shaped vertebrae (McLeod et al., 1964). As the number of carotid arteries severed is highly associated with neck gap size, it can be suggested that the “follow-through” stretch is paramount to causing the severing of one or more of the carotid arteries, and therefore reducing the blood supply to the brain and causing cerebral ischemia (Gregory and Wotton, 1990; Krause et al., 1988; Weir et al., 2002).

In terms of biosecurity and practicality, less external blood loss and skin tears are preferred in commercial environments (Galvin, 2005; Mumford et al., 2007), as well as being aesthetically more appealing. The likelihood of skin tearing was higher in unsuccessful kills, mainly attributed to the higher number of kill attempts and greater risk of over-stretching the neck. Interestingly, bird type or the confounded MCD method (“Ring” method) was more likely to be related to skin tearing, and was more likely to result in a lower dislocation level and fewer carotid arteries severed, suggesting that the “Ring” technique consistently performs sub-optimally in comparison to the “Two finger” method as well as the NMCD treatment. For both methods, skin tearing occurred more in cadavers than in live birds, again highlighting the difficultly in performing the treatments on a bird which has no muscle tone, and perhaps the lack of usefulness of cadaver practise for training.

The questionnaire revealed that less than half of stock-workers (3/8 stock-workers) considered the NMCD device as useful for dislocating bird’s necks, and only two stock-workers stated they preferred the device to MCD. Despite this, 50% of stock-workers stated they would consider using the NMCD device as an alternative to the now restricted MCD method, irrespective of their currently used MCD approach (“Two finger” or “Ring” method). Surprisingly, the stock-workers who said they would consider using the NMCD device did not have the highest performance rates in the method, but instead were the worst performers, but did perform well in MCD. The interpretation of this is difficult to determine; these stock-workers may be willing to consider the alternative as it is similar to MCD and not like other alternative methods (e.g. CPK), and therefore may be considered to be the next best thing. Since only half the stock-workers would consider it, this study demonstrates that a strong preference for MCD was present and the practice in application of the NMCD device was not enough to encourage stock-workers to consider an alternative method.

In conclusion, NMCD did not match the kill performance of MCD in a commercially relevant environment and did not completely remove variation in kill success and trauma generated by various users. However, the NMCD device was more likely to perform optimally when it was successful (e.g. severing carotid arteries, achieving C0-C1 dislocation) compared to MCD, suggesting it has promise if training could be optimised. Concerns were raised about the adaptability of stock-workers to use NMCD when their MCD method was not based on the “Two finger” method, and as there is no way to determine the scale of use of this technique in the UK poultry industry, it is difficult to judge the effect this may have on uptake and successful use of the NMCD method. Training requirements seemed to be sufficient at the basic level (a leaflet) for NMCD, and stock-worker performance improved with practice. Concern remains in terms of the willingness of stock-workers to consider an alternative method to standard MCD, even if their feedback from the trial’s questionnaire showed potential. The NMCD treatment requires further refinement and perhaps two training schemes would be most appropriate: one targeted at “Two finger” method experienced individuals and another aimed at completely inexperienced individuals, in order to optimise their performance.

8 General Discussion

The method by which poultry are killed on-farm is essential to the welfare of poultry flocks and individuals. This project set out to identify a new mechanical method for despatching poultry on-farm, to provide a replacement method for MCD. Under the new European Council Regulations on the Protection of Animals at the Time of Killing (1099/2009) (European Council, 2009), MCD has been heavily restricted as routine killing method, possibly due to concerns relating to its humaneness (i.e. time to loss of consciousness) (Carbone et al., 2012; Cartner et al., 2007; Erasmus et al., 2010a; Gregory and Wotton, 1990). In order to achieve this aim, the project had several objectives to ascertain preferences for, and requirements of, a mechanical method in order for it to be successful and have the potential to be supported by the poultry industry in the UK.

In Chapter 2, the results of a questionnaire demonstrated the high preference and routine use of MCD across the poultry stock-workers in the UK, although the reasons for this were not as clear or consistent across all individuals. This study also highlighted a lack of knowledge and unwillingness to consider an alternative killing method, irrespective of the European Regulation (European Council, 2009) restricting the use of preferred and currently used methods. The questionnaire did highlight the importance of a killing method meeting certain criteria, besides being effective (for example, being easy to use, time efficient, requiring minimal equipment, being inexpensive, and being humane). This information, as well as the evaluation of previous research, in Chapter 1 was used to design and prototype modifications to four mechanical devices which complied with the new European legislation (European Council, 2009) in an attempt to meet the majority of the highlighted criteria from the questionnaire. Two of the mechanical devices designed were focused around causing fatal brain damage, which previous research had suggested to be more humane than cervical dislocation methods (Erasmus et al., 2010a; Erasmus et al., 2010b; Finnie et al., 2000; Gregory et al., 2007; Raj and O'Callaghan, 2001). The remaining two devices were designed to cause cervical dislocation, despite concerns highlighted in previous research with regard to indicators of loss of consciousness suggesting that consciousness was not lost instantaneously with these methods (Carbone et al., 2012; Cartner et al., 2007; Erasmus et al., 2010a; Gregory and Wotton, 1990). When designing and modifying the methods, attempts were made to improve their effectiveness and ability to cause immediate loss of consciousness.

8.1 Evaluation of killing methods

The four mechanical methods were evaluated in three laboratory based experiments and the most successful device was then trialled in a commercial environment with multiple users in comparison with MCD. Throughout the laboratory studies, the NMCD was shown to be the most successful at killing birds (kill success $\geq$ 96% (Figure 8.1)) and demonstrated consistency in its application. As well as the ability to cause rapid loss of consciousness, shown through loss of reflexes and behaviours, birds killed with NMCD exhibited EEG spectral variables indicating unconsciousness (e.g. F50 < 12.7 Hz) significantly faster than the other methods. Unlike the other devices, the NMCD showed a consistently high kill success rate, irrespective of the state of the birds when it was applied (alive/dead or conscious/unconscious). All of the other killing devices showed a reduction in kill success as the studies progressed and birds were assessed in more realistic states, highlighting their inadequacies as killing methods in an on-farm context.

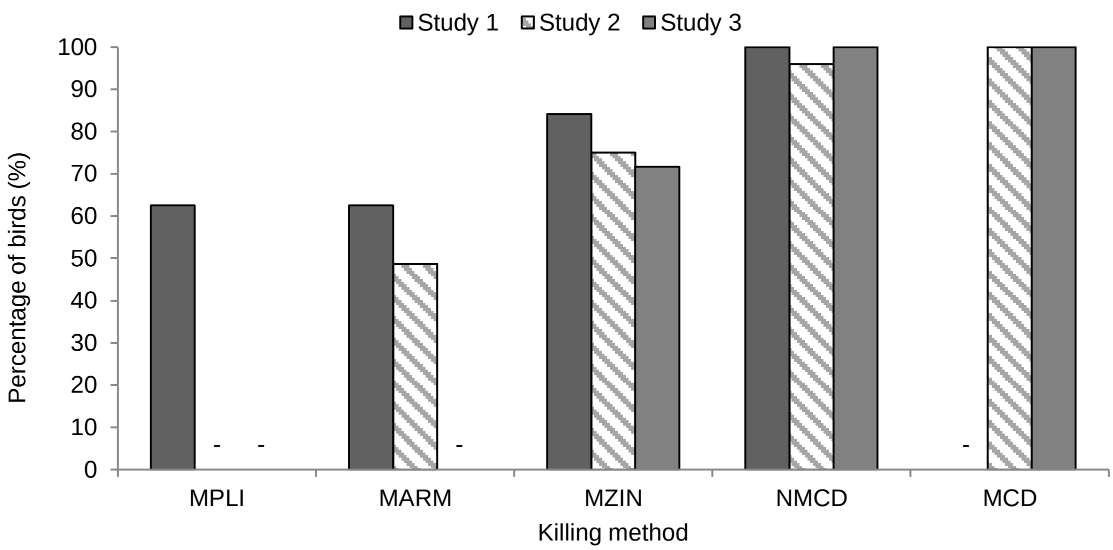


Figure 8.1 Comparison of kill efficacy across the three laboratory studies for each killing method. Studies marked with "–" indicate the killing method was not tested, for mechanical methods this represents when the method was dropped for further testing.

Device success (i.e. when the device had optimal/expected effect) was always lower than kill success (or killing potential), irrespective of killing method, and this was demonstrated across all three laboratory experiments and the on-farm trial. This indicates that the device success criteria may, in some cases, have been too strict and did not need to be fulfilled to achieve a successful kill. However, whether or not these criteria are fulfilled may have welfare consequences. The NMCD device was shown to be relatively consistent in live birds, either conscious or unconscious, with a device success rate of approximately 40%. Device success was significantly higher in cadavers (90%) compared to testing in live birds performed in later experiments, suggesting that the handling of live birds and compensation for their movements may limit device success. In all comparisons of device success, NMCD always out-performed MCD (across all experiments) and was more likely to cause optimal damage to the birds, including during the on-farm trial (Study 4, Chapter 7).This suggests that the NMCD had improved the consistency of the manual method and showed potential as a more reliable method for despatching poultry. Both the MCD and NMCD had relatively low device success rates, compared to the head trauma methods in live birds, however this may have been an artefact of their specific criteria for device success being higher and more detailed compared to the head trauma methods (refer to Table 4.2), which would have made achieving device success more difficult. This consistency in live birds was not seen in the MZIN or MARM, with device success rates decreasing as the experiments continued and the state of the birds reached more commercially realistic states.

In the fourth trial (Chapter 7), the most successful device in the three laboratory studies (NMCD) was tested in comparison with MCD in a commercial environment and with multiple users. The NMCD device did not match the kill performance of MCD in this setting (81.6% and 98.4%, respectively), and did not completely remove variation in kill success or effects on the anatomy across multiple users. However, NMCD was shown to reduce variation in the effect on anatomy within stock-workers and was associated with an improvement in their performance over time, showing potential for the device to be successful following further refinement in terms of training. The commercial trial also highlighted a significant issue in the adaptability of stock-workers to use NMCD if they were not trained in the “Two finger” MCD method technique. Thus, identifying a potential factor which could hamper the success and uptake of the NMCD method by the industry in the future, although as there are no records of how widely other MCD techniques are used, the scale of the potential issue is unknown. Helpfully, the “Two finger” technique is the recommended method for MCD by the HSA (HSA, 2004), therefore any companies (and their staff) which follow these guidelines should be less affected and better prepared for adapting to the NMCD method.

8.1.1 Cervical dislocation versus traumatic brain injury

The different strategies of killing methods for poultry determine their effect on the bird's anatomy and in turn how they cause brain death and loss of consciousness. Previous research has highlighted a preference for methods which cause direct trauma to the brain in other livestock species, as diffuse damage to brain tissue and neurons disturbs the normal electrical activity in the brain and can cause mass depolarization and neurogenic shock (Alexander, 1995; Anil et al., 2002a; Claassen et al., 2002; Kushner, 1998) which has been associated with rapid loss of consciousness and brain death. Conversely, methods which cause cervical dislocation attempt to isolate the brain from the rest of body and prevent blood flow, causing death by cerebral ischemia (Claassen et al., 2002; Kushner, 1998; Slivka et al., 1995; Weir et al., 2002; White and Krause, 1993).

To determine which strategy is the most effective on the methods can be evaluated in terms of success, humanness and practicality. In this project the NMCD device was shown to be the most humane (and only) mechanical cervical dislocation method, based on shortest latencies for EEG unconsciousness thresholds (F50 < 12.7 Hz), however the Modified Zinger (MZIN) (a brain trauma method) was shown to cause the shortest reflex durations, which are also used as indicators of consciousness. EEG activity could not be measured in MZIN treatment birds due to application issues, with the implant residing in the bolt path. Throughout the project there was a poor correlation between EEG and reflex data necessarily, and reflexes remained present for significantly longer than EEG activity. Therefore, even though the MZIN caused immediate loss in the majority of reflexes indicating brain death and/or loss of consciousness, there was a lack of EEG data to validate this, and the significantly lower kill success rate compared to NMCD resulted in the method being deemed less effective and reliable. However, previous research which compares a captive bolt to a cervical dislocation method has shown the opposite result (Bader et al., 2014; Erasmus et al., 2010a; Erasmus et al., 2010b; Gregory and Wotton, 1990), however those captive devices were specifically designed for poultry and therefore may have had an advantage (e.g. ease of aiming the device). Despite NMCD causing the shortest durations to F50 < 12.7 Hz and F50 < 6.8 Hz compared to other mechanical devices, it was not significantly different to durations for MCD, although the maximum range was considerably higher for MCD. However, it is open for debate as to whether this was humane enough. There are no published parameters on what is the acceptable duration between application of a killing method and loss of consciousness.

Ideally immediate loss of consciousness is the goal; however it is currently difficult to ascertain with the tools we have, particularly as EEG recordings are highly susceptible to artefacts during the application of a killing method (e.g. muscle contractions, body movements, impact of bolt, etc.) therefore there is a delay before the EEG trace is usable. It should be the aim to cause immediate or as close to immediate loss of consciousness of the animal for any killing method, therefore in the case of this project NMCD has shown an improvement to the currently used MCD method, with shorter latencies to isoelectric and lower maximums latencies to unconsciousness thresholds. The NMCD also proved to be more likely to consistently cause optimal or near optimal damage to MCD and with further development in its training it could provide a competitive and a more humane alternative to MCD.

The NMCD was also shown to be the most practical method, with minimal and inexpensive equipment required. The device was designed to be fairly intuitive to use for people experienced in MCD, although this was shown to be limited by which MCD technique the operator used. However, anecdotally, NMCD was easier to use than the MZIN and the MARM, which required time and additional help to position and secure birds prior to application, which in a commercial setting is not feasible. The NMCD was also shown to have the lowest biosecurity risk, with minimal numbers of birds releasing bodily fluids into the environment as a result of the device application, while for the head trauma methods, both resulted in the majority of birds receiving open wounds to the head and significant blood loss, which has been shown to a biosecurity issue and is not favoured in commercial environments (Halvorson and Hueston, 2006; Nerlich et al., 2009).

Applying cervical dislocation methods to birds which have leg or hip injuries/disease is a welfare issue. The majority of dislocation methods, which involve a stretch and twist action, require the bird’s legs to be held and act as an anchor for the operator to pull against in order to generate sufficient force to dislocate the neck. However if the birds’ legs and/or hips are damaged, inflicting strain onto the area is likely to cause pain and distress (Gentle, 2011; Gentle and Tilston, 2000; Murrell and Johnson, 2006; Whitehead and Fleming, 2000). This would be a particular issue for laying hens and broilers, who are susceptible to osteoarthritis and other degenerative skeletal diseases (Anderson-

Mackenzie et al., 1997; Julian, 2005; Whitehead and Fleming, 2000). This issue is not applicable to head trauma methods such as MZIN and MARM, which only require the body of the bird to be restrained by another operator (however, the process of handling the bird for these methods is also stressful and should be minimised) (Chambers et al., 2001; Gregory, 1994; Kettlewell and Mitchell, 1994; Petracci et al., 2010; Schilling et al., 2008; Scott, 1993). However, this apparent advantage with head trauma methods needs to be considered in the context of the generally lower kill success rates of these devices, as demonstrated in this project.

Other factors which affected the efficacy of the killing methods were bird type, bird age and bird weight, although these individual factors were occasionally confounded with one another, making individual analysis of their effects difficult to ascertain. In cadaver birds, these factors were shown to have no effect on the kill potential of devices which caused head trauma, but were shown to have an effect on cervical dislocation methods, with both younger/lighter and broiler type birds being related to higher kill potentials compared to older/heavier and layer type birds. However, the effect of these external factors was not consistent. In live birds (unconscious or conscious), bird type and bird age had the most effect on the effectiveness of the MARM and MZIN compared to the NMCD and MCD, and demonstrated the limited ability of these devices to adapt to individual bird variation and bird movements (i.e. behaviour), despite modifications. However, bird characteristics also had an effect on cervical dislocation devices too, highlighting the challenge for any killing method is to be applicable to all bird types, age and weights; and perhaps attempting to develop one method for every context may not be appropriate. The results of Studies 2 and 3 (Chapters 5 and 6), as well as the on-farm trial (Chapter 7) showed that the NMCD was the device most likely to cause optimal damage, irrespective of these bird factors and it out-competed MCD in terms of post-mortem evaluations and device success.

8.2 Evaluation of methodology

8.2.1 Limitations of EEG

The use of EEG activity as an indicator of electrical brain activity is well documented (Delorme and Makeig, 2004; Haberham et al., 1999; Haberham et al., 2008; Lowe et al., 2007; McKeegan et al., 2011; Pallis and MacGillivray, 1980; Tidswell et al., 1987).

However, it does have limitations in terms of interpretation and measurement. There is limited research on EEG parameters defining conscious states in birds (Sandercock et al., 2014), and many are subjectively defined by visual evaluation of the raw trace to identify isoelectric state (brain death), VERs, and the increase of slow wave activity (Erasmus et al., 2010a; Gregory and Wotton, 1990). In this project, EEG recordings were used in Study 2 to evaluate time to loss of consciousness. The EEG electrodes used rested on the surface of the dura (technically an electrocortigram) and therefore only measured the electrical potentials on the surface of the cerebral cortex, and could not measure activity deeper in the brain (e.g. brain stem), which may have been more indicative of wider brain function (Delorme and Makeig, 2004; Haberham et al., 2008; Pallis and MacGillivray, 1980; Verhoeven et al., 2014). As stated previously, consciousness cannot be directly measured, but we can infer it from changes in the EEG activity, and subtle changes in the trace are difficult to define (Alkire et al., 2008; Verhoeven et al., 2014). For example the F50 < 12.7 Hz was defined as the threshold for unconsciousness in this study, however it represents a point at the higher end of a gradient, indicating a conscious state somewhere between sedated and fully unconsciousness, as indicated by previous research (Sandercock et al., 2014). Potential anaesthetic effects were present in Study 2 as they birds were lightly anesthetised immediately prior to testing to protect their welfare. Therefore assessment of the birds' conscious states during and immediately post-killing must take into account the possibility of anaesthetic effects being present, although analysis was designed to minimise these effects and prevent birds being wrongly termed unconscious as a result of the killing device, when it may have in fact been due to anaesthetic.

Another issue with EEG data is large individual variation as well as inter-species variation, making validation of parameters difficult. Thus the margin for error was large, although the large sample size and continuous assessment of the EEG trace for each bird compensated for this. The final issue with EEG is the risk of artefacts within the trace, which can invalidate their use. These can be caused by technical issues (e.g. cable movement, mains noise hum) or by the animal itself (e.g. muscle contractions or eye movement) (Alkire et al., 2008; Delorme and Makeig, 2004; Gwin et al., 2010; Haberham et al., 2008; Verhoeven et al., 2014). These factors accounted for a loss of approximately 38% of epochs during Study 2 (Chapter 5). The use of the novel filtering method developed as part of Experiment 2 significantly reduced the loss of epochs, however, there is currently no method for compensating for severe movement artefacts in the trace, which are likely to occur around the time of killing due to the clonic convulsions or the risk of the implant being dislodged as a result of the killing method (Becker et al., 2010; Coenen et al., 2009; Delorme and Makeig, 2004; Gwin et al., 2010; Lowe et al., 2007; McKeegan et al., 2013b; Sandercock et al., 2014; Simons et al., 1989).

8.2.2 Limitations of reflex and behaviour measures

The key issue with using reflexes and behaviours as indicators of conscious state was that they did not correlate well with EEG activity; reflexes and behaviours remained present for significantly longer than expected based on spectral variables indicating unconsciousness. Previous research has also shown this inconsistency (Erasmus et al., 2010a; Erasmus et al., 2010c; Gregory and Wotton, 1990; Sandercock et al., 2014) has used this as an advantage, suggesting that the loss of reflexes and behaviours is therefore a highly conservative measure of loss of consciousness and brain death. When more accurate EEG measurements are not possible (e.g. in commercial settings) measuring reflexes and behaviours is often more practical and can still be informative as a relative measure between killing methods.

During this project, the frequency of measurements for reflexes and behaviours was lower than desired; however due to the number of reflexes/behaviours measured it was not possible to record them more frequently than at 15 s intervals. This did however reduce the accuracy of determining when the reflexes were lost, and led to over-estimation of them (which again represents a highly conservative measure which did not infer loss of consciousness prematurely). Another important issue with the use of reflexes and behaviours as indicators of brain death and loss of consciousness was that sometimes they were difficult to assess (e.g. damaged eyes made the pupillary and nictitating membrane reflexes hard to identify or clonic convulsions made observations of rhythmic breathing difficult to assess) (Anil, 1991; Croft, 1961; Erasmus et al., 2010c; Prinz et al., 2012; Sandercock et al., 2014; Shaw, 1989).

8.2.3 Limitations of post-mortem evaluations

The post-mortem evaluations of the trauma caused to the birds' anatomy as a result of the killing method acted as a marker for the severity of caused. These could also be used to infer effects on the birds' likelihood of consciousness. Previous research has documented how various forms of trauma cause both primary (e.g. lacerations) and secondary (e.g. changes inter-cranial pressure, biochemical disruption) stage injuries, which, when directed at the spinal cord or the brain (including the brain stem), resulted in disruption of electrical activity of axons and brain tissue, and functional impairment (Alexander, 1995; Anderson et al., 2006; Brieg, 1970; Dumont et al., 2001a; Kushner, 1998; Povlishock et al., 1992; Shi and Pryor, 2002; Takahashi et al., 1981; White and Krause, 1993).

As the project progressed, attempts were made to improve the measurements recorded as part of the post-mortem evaluations, either by recording additional measures or increasing the detail and accuracy of measures. In Experiment 1 (Chapter 4) post-mortem measures were mainly restricted to binary yes/no recordings for damage to different tissue regions, e.g. spinal cord severed (yes/no), left forebrain damaged (yes/no). However, assessment of the methodology following the completion of the experiment highlighted the need for greater detail and recording of the location of damage to the skull, rather than a crude binary measure of whether or not it was damaged. These improvements were taken forward to the following experiment (Chapter 5), and improved the detail with which the physiological trauma could be analysed and related to the method success rates. It was within this that data revealed that with head trauma methods, birds were more susceptible to damage on one side of the head as a result of the operator's handedness (e.g. operator was left-handed, and this resulted in a right-side bias in brain and skull damage). This side bias for damage was an undesirable effect on the birds' anatomy and highlighted another issue with the MZIN and the MARM, which was not displayed in cervical dislocation methods. Following Experiment 2, it was determined that, if possible, a more detailed assessment of the type of damage/injury sustained to each brain region was required to more accurately show differences between successful and unsuccessful kills, as well as between devices. As a result a basic grading system was implemented in Experiment 3 in order to differentiate between minor, medium, and severe damage to each brain regions (refer to Table 6.3).

8.3 Future work

As the NMCD device has shown such potential it would nice to follow on from this work and consider evaluating training methods in more detail, as well as incorporating a social science side in order to take into account more stock-worker information (e.g. experience and education). It has also been proposed to develop the device further and expand its efficacy evaluation to other bird types (e.g. turkeys and broiler breeders), where the need for an alternative method is also a priority to the industry.

Furthermore, as all the studies which included MCD demonstrated that previous concerns in terms of its kill efficacy and time to unconsciousness may not be fully warranted, it would be a logical step to compare MCD and NMCD in live and conscious birds, while recording both EEG and ECG data, which if funding had been available would have been included within this thesis.

8.4 Conclusions

This series of experiments have identified a potential new mechanical killing device for despatching poultry on-farm, the NMCD device. The device consistently killed birds and caused rapid loss of consciousness. The NMCD device matched the performance of MCD in the laboratory trials, however in the on-farm study; issues were identified with training and adaptability dependent on the stock-worker’s experience. However, it did show potential and stock-workers improved over time. Further refinement is needed in terms of appropriate training for NMCD. Collectively, the results suggested that the NMCD device has the potential to be developed into a marketable product which could be made available to the poultry industry as well as back-yard poultry keepers.

Appendix 1 Survey circulated to the members of the British Poultry Council (BPC).

Survey of preferred culling methods for poultry on farm.

Please return the completed survey to the address below by 20th February 2012:

Jessica Hopkins

Avian Science Research Centre

SAC Auchincruive

Ayr

KA6 5HW — Or by email to: Jessica.Hopkins@sac.ac.uk

1. Employing Company: ____

2. Main poultry species: ____

3. Poultry experience: ____________ Years ______ Months

4. Gender *(please circle)*: Male Female

5. Which killing method is the normal procedure at your work place for dispatching sick, injured, or runt birds?
(Please circle one)

Neck dislocation by hand	Cartridge-powered percussive device (e.g. CASH Poultry Killer)	Neck dislocation by Broomstick	Pneumatic percussive device (e.g. CASH air powered poultry killer)	Pliers e.g. Semark
Decapitation	Electrical Stun/kill	Other (Please state):	______________________	

6. Which is your preferred method for dispatching sick, injured, or runt birds?
(Please circle one)

Neck dislocation by hand	Cartridge-powered percussive device (e.g. CASH Poultry Killer)	Neck dislocation by Broomstick	Pneumatic percussive device (e.g. CASH air powered poultry killer)	Pliers e.g. Semark
Decapitation	Electrical Stun/ kill	Other (Please state):	______________________	

7. Reasons for your preference:
(Please circle as many as required)

time efficient	humaneness	easy to use/learn	cost efficient	low maintenance

high success rate in killing birds on first application	lower operator fatigue risk	good operator health and safety

Other (Please state): ____

8. Can you suggest any improvements to your preferred method?

____

____

____

9. Are there circumstances when you would consider the use of a mechanical device/aid to be more appropriate than the normal procedure or your preferred method for killing sick, injured, or runt birds?*(Please circle)*

Yes No

10. Please explain your answer to question 9.

____

____

____

Thank you for completing this survey.

<u>Appendix 2</u> Survey circulated to the members of the British Egg Industry Council (BEIC).

Supporting the land-based industries for over a century
SAC

Survey on Preferred Killing Methods for Poultry

1. Employing Company: ______________________________

2. Main production species of company: ______________________________

3. Poultry experience: ____________ Years ______ Months

4. Gender *(please circle)*: Male Female

5. Which killing method is the normal procedure at your work place for dispatching sick, injured, or runt birds?
(Please circle one)

Neck dislocation by hand	Cartridge-powered percussive device (e.g. CASH Poultry Killer)	Neck dislocation by Broomstick	Pneumatic percussive device (e.g. CASH air powered poultry killer)	Pliers e.g. Semark
Decapitation	Electrical Stun/kill	Other (Please state):	______________	

6. Which is your preferred method for dispatching sick, injured, or runt birds?
(Please circle one)

Neck dislocation by hand	Cartridge-powered percussive device (e.g. CASH Poultry Killer)	Neck dislocation by Broomstick	Pneumatic percussive device (e.g. CASH air powered poultry killer)	Pliers e.g. Semark

Decapitation | Electrical Stun/ kill | Other (Please state): ______________________

7. Reasons for your preference:
(Please circle as many as required)

time efficient | humaneness | easy to use/learn | cost efficient | low maintenance

high success rate in killing birds on first application | lower operator fatigue risk | good operator health and safety

Other (Please state): ______________________________________

8. Can you suggest any improvements to your preferred method?

____

____

____

9. Are you aware of the changes to the EU regulations on killing poultry coming into force in 2013 (i.e. manual cervical dislocation (MCD) can only be performed on birds up to a live weight of 3kg and only 70 birds may be killed by MCD per stock-worker per day)? ***(Please circle)***

Yes No

10. Will the new EU regulations affect your future killing method choice due to:

(Please circle)

a) Bird weight? Yes No

b) Total killing number limit per stock-worker per day? Yes No

Thank you for completing this survey.

Jessica Hopkins

Avian Science Research Centre

SAC Auchincruive

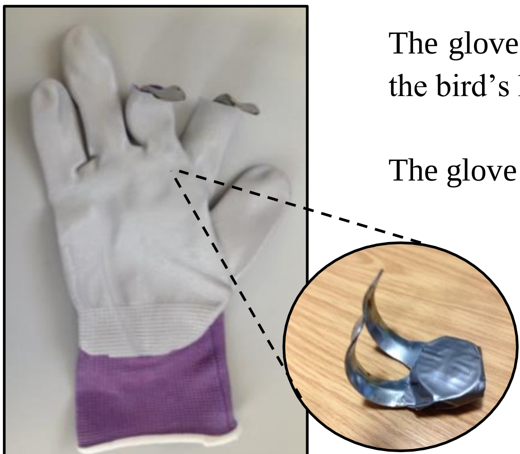
Ayr

KA6 5HW Or by email to: Jessica.Hopkins@sac.ac.uk

Appendix 3 NMCD Training Leaflet

Cervical Dislocation Glove

The cervical dislocation glove is a device to kill chickens on-farm. In essence it makes manual cervical dislocation (i.e. necking by hand) a mechanical method, with the use of the glove to aid the application.



The glove should be worn on the hand which will hold the bird's head.

The glove should be tight fitting and pulled on fully.

Your index and middle fingers rest above the finger supports and the metal edge between them should be parallel with the fleshy skin between your fingers.

Place the metal fingers around the birds head – the metal fingers move to fit different sizes of bird.

The tips of the metal fingers should rest under the bird's jaw and the metal edge (between the metal fingers) should rest behind the bird's skull, at the top of the neck.

Close your thumb, ring and little finger under the bird's head to secure it in place.

Same technique as manual cervical dislocation!

With your un-gloved hand hold the bird's legs and rest its underside against your thigh.

In one swift movement stretch the bird's neck downwards, while rotating the bird's head back towards the ceiling and forcing the metal edge into the back of the bird's head/top of the neck.

Check for signs of bird death:

- a gap in the neck
- no breathing or eye reflexes present

Mechanical on-farm killing device designed in accordance of the European Directive (EU 1099/2009) and Scottish Regulations on the Welfare of Animals at the Time of Killing (2012). Funded by the Humane Slaughter Association (HSA).

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